Migration and spread of drug resistant tuberculosis (DRTB) in Zimbabwe

By

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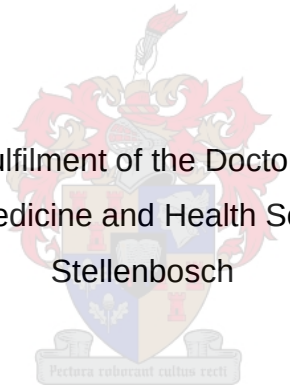

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that I have not previously in its entirety or in part submitted it to any University for purposes of obtaining a degree

Name …….. …….

Signature…….. Date ……..

The dissertation includes 2 published papers in peer reviewed journals and 4 unpublished papers. The development and writing of the papers (published and unpublished) were the principal responsibility of myself, and for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extend of contribution of the co-authors.

Abstract

The Southern African Development Community (SADC) is characterised by extreme poverty, malnutrition, high human immunodeficiency virus (HIV) prevalence in the adult population and weak health systems. These factors promote transmission of tuberculosis (TB), rifampicin resistant and multidrug resistant (RR/MDR)-TB. Countries with high TB incidence in the SADC region were, South Africa (768/100,000), Namibia (729/100,000), Botswana (360/100,000), Lesotho (411/100,000) and Swaziland 854/100,000 population. Zimbabwe, with an estimated TB incidence of 221/100,000 shares poorly controlled borders with two of her highly burdened neighbours, South Africa and Mozambique. Although the World Health Organization (WHO) estimated RR/MDR-TB prevalence for Zimbabwe was 1,500 per year, the country reported 406 cases in 2018. The drug resistance survey (DRS) estimated that the RR-TB prevalence was 4% among new patients and 14.2% among retreatment cases in 2016. Since the country started providing RR/MDR-TB diagnosis, treatment and care, the maximum reported cases were just above 500 cases in 2014, an indication of low RR/MDR-TB case finding.

An estimated three million Zimbabweans are believed to be immigrants in SADC countries. The paucity of evidence on cross border migration and RR/MDR-TB transmission in high burden countries motivated the study to describe the contribution of human migration to the spread of DR-TB in Zimbabwe.

We reviewed published literature on migration and spread of DR-TB at both global and regional level. A geospatial analysis study of TB in Harare city aimed to assess whether environmental conditions similar to those faced by immigrants could promote TB transmission. Routinely collected and stored RR-TB isolates were spoligotyped

and whole genome sequenced (WGS) to estimate presence of strains that had never been reported in Zimbabwe, defined as foreign strains. A phylogeographic study of the DR-TB Lineage 2 (L2) between South Africa and Zimbabwe aimed to explain the presence of DR-TB L2 strains in Zimbabwe.

Evidence of RR/MDR-TB active transmission under migration settings was minimal. DR-TB disease was confined to immigrants with the risk falling after 5 years of stay. Few studies from high RR/MDR-TB burden settings did not show evidence of active transmission. Transmission potential from high burden countries was possible given the associated poverty, high HIV prevalence and high mixing patterns between immigrants and natives.

Epidemiological analysis using geospatial techniques showed that high transmission patterns were confined in one health district with a high population of internally displaced people and limited access to health services. Understanding transmission patterns may assist in planning interventions in high burden settings where resources are scarce.

The recovery of more than 60% of *Mycobacterium* tuberculosis (Mtb) isolates was the first description of long term storage at room temperature in low income countries. This could change the scope of TB research as the currently recommendations of minus 70^0C are not readily available. Drug resistant-TB isolates from Zimbabwe showed the predominance of the L2 strains, 45/184 (24.5%). Compared to previously reports of zero percent and 12% in 2007 and 2011 respectively, this was a significant increase. The DR-TB L2 strains were mainly confined to the southern part of Zimbabwe and northern provinces of South Africa. The Zimbabwean southern province has the highest HIV prevalence rate and strong historical cultural linkages with South African northern provinces.

Phylogeographic analysis did not show conclusive results on directional spread of DR-TB L2 strains between Zimbabwe and South Africa despite presence of time and space clustering. Pre-existing Bedaquiline and Delamanid resistance markers of Zimbabwean isolates was disturbing given the importance of these drugs in the proposed new shorter regimens.

Although our findings could not categorically demonstrate spread of DR-TB L2 between South Africa and Zimbabwe, the findings provided the first evidence on possible migration related transmission in high burden settings. Our findings may have been affected by presence of re-infection in this high burden settings. We strongly recommend a regional cross border surveillance and treatment project using WGS for diagnosis and contact investigation. The pharmaceutical industry in South Africa and Zimbabwe must work together to develop new anti-tuberculosis drug molecules and respond to the unique drug resistance patterns circulating in the region.

Opsomming

Die Suider-Afrikaanse Ontwikkelingsgemeenskap (SAOG) word gekenmerk deur armoede, wanvoeding, hoë voorkoms van menslike immuniteitsgebreke (MIV) in die volwasse bevolking en swak gesondheidstelsels. Hierdie faktore bevorder die oordrag van tuberkulose (TB), rifampisien-weerstandige en multi middelweerstandige (RR / MDR) -TB. Lande met 'n hoë voorkoms van TB in die SAOG-streek is Suid-Afrika (768 / 100.000), Namibië (729 / 100.000), Botswana (360 / 100.000), Lesotho (411 / 100.000) en Swaziland 854 / 100.000. Zimbabwe, met 'n geraamde TB-voorkoms van 221 / 100,000, het swak beheerde grense met twee hoë TB voorkoms buurlande, Suid-Afrika en Mosambiek het. Alhoewel die Wêreldgesondheidsorganisasie (WGO) beraam dat RR / MDR-TB-voorkoms vir Zimbabwe 1 500 per jaar is, het die land in 2018 406 gevalle gerapporteer. Die medisyne-weerstandige opname (DRS) het geskat dat die RR-TB voorkoms 4% onder nuwe pasiënte was. en 14,2% onder gevalle van herbehandeling in 2016. Sedert die land begin met die diagnose, behandeling en versorging van RR / MDR-TB, was die maksimum aangemelde gevalle in 2014 net meer as 500 gevalle, 'n aanduiding van die lae RR / MDR-TB-bevindings.

Na raming is daar 3 miljoen Zimbabwiërs immigrante in SAOG-lande. Die min bewyse oor grens migrasie en oordrag van RR / MDR-TB in hoë las TB lande, het hierdie studie gemotiveer om die bydrae van menslike migrasie tot die verspreiding van DR-TB in Zimbabwe te beskryf.

Ons het gepubliseerde literatuur van migrasie en verspreiding van DR-TB op wêreld- and streeksvlak nagegaan. 'n Geografiese-ontledingstudie van TB in Harare is gedoen om te bepaal of omgewingstoestande soortgelyk aan immigrante TB-oordrag kan bevorder. RR-TB-isolate wat gereeld versamel en gestoor is, is gespoligotipeer en die

hele genoom volgorde bepaling (WGS) is gedoen, om die teenwoordigheid te bepaal van TB-stamme wat nog nooit in Zimbabwe aangemeld is nie, gedefinieer as vreemde stamme. 'n Filogeografiese studie van die DR-TB Lineage 2 (L2) tussen Suid-Afrika en Zimbabwe is gedoen om die teenwoordigheid van DR-TB L2-stamfamilie in Zimbabwe te verklaar.

Bewyse van aktiewe transmissie van RR/MDR-TB onder migrasie-omgewings was minimaal. Infeksie met DR-TB was beperk tot immigrante met die risiko dat dit na vyf jaar van verblyf daal. Min studies met hoë RR / MDR-TB voorkoms areas is gedoen en het geen bewys van aktiewe transmissie getoon nie. Die oordragpotensiaal van lande met 'n hoë las was moontlik vanweë die gepaardgaande armoede, hoë MIV-voorkoms en 'n hoë mengpatroon tussen immigrante en inboorlinge.

Epidemiologiese ontledings met behulp van geografiese tegnieke (GIS) het getoon dat hoë transmissiepatrone in een gesondheidsdistrik beperk was met 'n hoë bevolking van mense wat binnelands verplaas is en beperkte toegang tot gesondheidsdienste het. As transmissiepatrone verstaan word, kan dit help met die beplanning van intervensies in areas met 'n hoë TB voorkoms waar hulpbronne skaars is.

Die terugwin van meer as 60% van *Mycobacterium tuberculosis* (Mtb) isolate was die eerste beskrywing van langtermyn berging by kamertemperatuur in lande met lae inkomste. Dit kan die omvang van TB-navorsing verander, aangesien die huidige aanbevelings van -70°C nie geredelik beskikbaar is nie. Middelweerstandige TB-isolate uit Zimbabwe het die oorheersing van die L2 -TB stamfamilie, 45/184 (24,5%), getoon. In vergelyking met voorheen verslae van onderskeidelik 2007 en 2011 met nul persent en 12%, was dit 'n beduidende toename. Die DR-TB L2 TB stamfamilie was hoofsaaklik beperk tot die suidelike deel van Zimbabwe en die noordelike provinsies van Suid-Afrika. Die Zimbabwiese suidelike provinsie het die hoogste

voorkomssyfer van MIV en sterk historiese kulturele bande met Suid-Afrikaanse noordelike provinsies.

Filogeografiese ontledings het nie onweerlegbare resultate oplewer oor die rigtingverspreiding van DR-TB L2-stamfamilie tussen Zimbabwe en Suid-Afrika nie, ondanks die feit dat tyd en ruimte saamgroepeer. Die bestaande Bedaquiline- en Delamanid-weerstandmerkers van Zimbabwiese isolate was ontstellend, gegewe die belangrikheid van hierdie middels in die voorgestelde nuwe korter behandelings regimente.

Alhoewel ons bevindinge nie die verspreiding van DR-TB L2 tussen Suid-Afrika en Zimbabwe kon demonstreer nie, het die bevindings die eerste bewys gelewer oor moontlike migrasieverwante transmissie in hoë lasomgewing. Ons bevindings is moontlik beïnvloed deur die teenwoordigheid van herbesmetting in hierdie hoë TB las areas. Ons beveel sterk aan dat 'n plaaslike oorgrens toesig- en behandelingsprojek wat gebruik maak van WGS gebruik word vir diagnose en kontakondersoek. Die farmaseutiese industrie in Suid-Afrika en Zimbabwe moet saamwerk om nuwe geneesmiddels teen tuberkulose te ontwikkel en te reageer op die unieke middelweerstandigheidspatrone wat in die streek sirkuleer.

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Chapter 1

General Introduction

1.1 Global and African region burden of TB and MDR-TB

Tuberculosis (TB) is an ancient disease caused by *Mycobacterium tuberculosis* (*Mtb*) bacteria transmitted from person to person through droplet nuclei (1). Despite the global decline in TB notification rate, the World Health Organization (WHO) African region and South East Asia remain highly burdened by the TB epidemic (2) (Figure 1). Although the estimated global total cases have remained stable at about 10 million per year, there was an improvement in the number of notified new TB cases from 6.4 million (64%) cases in 2017 to 7 million (70%) cases in 2018 (3). This may be an indication of continued transmission in the presence of intensified TB prevention and control activities. Limited resources, conflict, general instability and a high HIV infection prevalence contribute to the high TB incidence in Sub Saharan Africa and South East Asia sub regions (4).

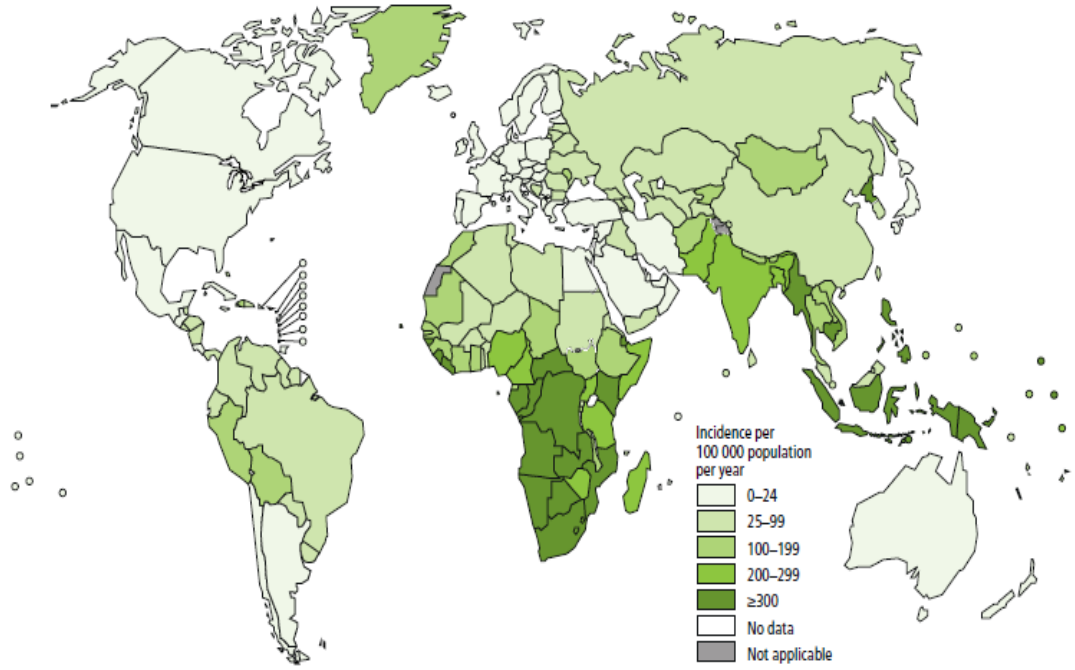

Figure 1 Global estimated TB incidence rates, 2017 (5)

Source: With permission, WHO Global tuberculosis report, 2018

Four SADC countries appear in all three TB, TB/HIV and MDR-TB high burden countries, namely, Democratic Republic of Congo (DRC), Mozambique, South Africa and Zimbabwe. Six countries, including Zimbabwe, from the SADC region have shown significant and consistent 4-8% decline in TB incidence from 2013-2017(5). Despite this decline in TB incidence to 221/100,000 in Zimbabwe, the country reported only 474 out of an estimated 1,300 rifampicin resistant (RR)/MDR-TB cases in 2017 (2). Treatment success for RR/MDR-TB patients in Zimbabwe was low, 51% with a high mortality rate of 20% in 2017 (2). This severe under reporting of RR/MDR-TB cases was due to the reduced access to diagnostic laboratory services (6). Tuberculosis incidence in the five most common migrant receiving countries in the SADC, namely, South Africa (768/100,000), Namibia (729/100,000), Botswana (360/100,000), Lesotho (411/100,000) and Swaziland 854/100,000 population is high (SADC TB Annual Report, 2009).

Migration and spread of MDR-TB in the SADC region

The contribution of migration to the global spread of MDR-TB has been described in the context of people moving from high TB burden to low burden settings (7,8). In addition to MDR-TB infected individuals transmitting infection during and after movement, migration also increases the vulnerability to TB infection, disease progression and poor treatment outcome (9–11). African region migration has remained largely intra-continental with only the more skilled persons able to migrate out of Africa in search of better livelihoods (12,13). Within the SADC region migration dates back to the late 1800 when males from rural areas moved into urban mining towns of South Africa to work in mines (14). Cross border migration initially into South Africa as part of mine labour migrants but later into Zimbabwe to work in both mines and farms around early and mid-1900 (15). Few studies have described the migration-

associated spread of MDR-TB between high burden countries in East Africa, Asia and Southern Africa (14,16,17). In Southern Africa, the studies have been limited to drug sensitive TB and among people with a history of working in mines (14,18).

Over the last two decades, Zimbabwe experienced severe socioeconomic challenges that resulted in her citizens emigrating to neighbouring countries in search of livelihoods (19). About three (3) million Zimbabweans are believed to be living in South Africa alone, with a mixture of documented and undocumented immigrants(20). These legal/documented and illegal/undocumented migrants frequently travel to and from Zimbabwe during festive season and in cases when they fall ill from HIV and AIDS. During these frequent movements, they continue to be at risk for contracting TB because of reduced access to health care services, over-crowding and inadequate nutrition (21). The added effects of poverty, HIV infection and poor TB treatment outcomes, further increases the risk of TB spread during and after the migration process (22).

The paucity of evidence on cross border migration and RR/MDR-TB transmission in high burden countries has been due to inadequate research capacity (21). Transmission of MDR-TB assessment uses a combination of epidemiological linkages, time of diagnosis and molecular techniques (7,23). Molecular techniques used to detect transmission during migration have not been readily available in high burden settings. In addition, the very high numbers of patients from high burden settings make follow up of patients and their contacts prohibitively resource intense. Geographic information systems (GIS) are able to describe spatiotemporal relatedness of cases and presence of hotspot distribution but are not able to confirm transmission of a specific strain from one person to the other (24).

Statement of the problem

Zimbabwe National TB Control Programme (NTP) started providing treatment of drug resistant TB under programmatic conditions in 2010. The NTP notified and enrolled an average of more than 400 RR/MDR-TB cases per year, with WHO estimating that the programme was not able to find all cases of RR/MDR-TB and therefore under-reporting the true burden of disease (2). Increased mortality of drug susceptible TB patients during treatment, from the southern region compared to the North may have been due to the missed RR/MDR-TB cases (25). Risk factors for mortality were recurrent TB, HIV co-infection and above 65 years of age (26). Given the low access to second line drug sensitivity testing (DST) services among TB patients in Zimbabwe, the likelihood of retreatment cases being missed RR/MDR-TB could have been high, hence high mortality (6). The Southern region population has strong cultural linkages with Botswana and South Africa and more people from the southern parts of Zimbabwe tend to migrate into neighbouring countries more than those from the northern part (19). Because of the increased movement across the borders of South Africa and Zimbabwe, high TB mortality from the southern region, inadequate laboratory system for second line culture and drug sensitivity testing (CDST), we hypothesized that imported RR/MDR-TB strains were circulating in Zimbabwe, more from the southern part of the country and propagating RR/MDR-TB transmission (8),(6,26). We used the prevalence of the RR/MDR-TB Lineage 2 (L2) in Zimbabwe, a strain that had not been reported in the country, to estimate the influence of migration on the epidemic (27,28). The key question was whether there was ongoing cross border transmission of RR/MDR-TB between Zimbabwe and her SADC neighbours? This study aimed to describe the molecular epidemiology of drug resistant *Mtb* in Zimbabwe and the influence of migration on the epidemic.

1.2 Study hypothesis

The increasing RR/MDR-TB L2 strain population in Zimbabwe from 0% in 2007 and 12% in 2011 is due to cross border migration between Zimbabwe and her neighbours

1.3 Specific Study Objectives

1.3.1 To describe the epidemiology of drug resistant TB in Zimbabwe

1.3.2 To describe the available evidence on the epidemiology of migration-related MDR-TB

Hypothesis: There is active MDR-TB transmission between migrants and local people in high TB and MDR-TB burden countries.

1.3.3 To demonstrate that presence of intense transmission areas or hotspots, are responsible for the propagation of TB transmission in high TB and MDR-TB burden settings.

Hypothesis: TB occurs in epidemic form, and the hotspot maybe driving the TB epidemic in Harare City

1.3.4 To describe the molecular epidemiology of MDR-TB patients notified between 2011 to 2016 in Zimbabwe

Hypothesis: Migration between Zimbabwe and the SADC region had increased the circulation of *Mtb* strains previously not reported (foreign) to Zimbabwe

1.3.5 To describe the transmission patterns of RR/MDR-TB Lineage 2 (L2) due to migration between South Africa and Zimbabwe

Hypothesis: Migration between South Africa and Zimbabwe has modified the RR/MDR-TB L2 strains population in Zimbabwe

1.4 Structure of the Thesis

Each of the chapters is structured for potential publication. For published articles, the format of the publishing journal was used. For unpublished papers, the formatting use in this dissertation was used, including references.

Chapter 1. Chapter 1 provides the general introduction to the thesis highlighting the key knowledge gaps in migration and transmission of MDR-TB in high TB/MDR-TB burden countries. Challenges in generating evidence on migration and transmission of MDR-TB in high burden settings are also highlighted. The problem statement, objectives of the thesis and structure are presented at the end of the chapter including the layout of the thesis indicating the key results for each of the chapters.

Chapter 2. The chapter was structured for publication in the journal of Tuberculosis. My contribution in this paper includes the design, analysis and writing of the manuscript. It summarizes the contribution of migration on TB, the gaps on migration-related TB transmission in high burden settings and appropriate technologies available to describe epidemiology of TB associated with migration. Objective 1 is addressed through Chapter 2, a narrative literature review on MDR-TB occurrence and migration. The chapter provides evidence on cross-border migration between high and low TB burden countries, between two high TB burden countries and intra-country migration. It also highlights that migration does not only contribute towards transmission of acute infectious diseases like the swine flu virus, but also affect the epidemiology of chronic infectious diseases like TB (29).

Chapter 3. Chapter 3 provides the burden of the RR/MDR-TB situation in Zimbabwe, as a published article. My major contribution to the article was in data analysis and review of the manuscript during the publication process including responding to the

reviewers' comments. In addition, I was the team leader responsible for report writing of the drug resistance survey report for the Zimbabwe National Tuberculosis Programme (see addendum 1). The publication showed the burden of RR/MDR-TB was 4.6% among notified new patients and 14% among notified retreatment cases. The results of this facility-based cross-sectional survey confirmed that the country was reporting less than the estimated RR/MDR-TB cases, an indication of low access to diagnostic services.

Chapter 4. Chapter 4 describes the epidemiology of TB disease in settings with high internal migration. The manuscript highlights the ability of the *Mtb* to occur in epidemic form and the contribution of TB hotspots in the propagation of transmission in the general population. My contribution in this paper included design of the study and writing of the manuscript. My co-authors from the Department of Geography at the University of Zimbabwe were responsible for the data collection, analysis and drawing the spatial maps in Geographical information systems (GIS) software. We used secondary data from Harare City, the capital city for Zimbabwe that was known to contribute the largest case load of TB in Zimbabwe. The chapter highlights the importance of ensuring availability of health care services in peri-urban poor settlements as these become TB hotspot and contribute to general TB epidemic as previously described by Dowdy et al in Brazil (30) Objective 2 is addressed in chapter 4, which highlights the effects of social and environmental conditions similar to those found during migration processes, on the transmission of *Mtb*. During migration, there is overcrowding and reduced access to health services, factors that have been demonstrated as conducive for active transmission of *Mtb* (21). The importance of this chapter is the contribution towards the strengthening of the programmatic

management of drug resistant TB in Zimbabwe to enable targeted interventions and understand the transmission dynamics of MDR-TB.

Chapter 5. Chapter 5 describes the methods used to recover *Mtb* bacteria from stored TB-positive liquid culture isolates. This published protocol paper, proposes a low-cost storage of *Mtb* isolates in liquid culture media for up to 6 years. Current protocols recommend freezing of samples at sub-zero temperatures. The key finding in this paper has the possibility of changing the protocol for recovering stored isolates for research purposes. Findings from this paper have implications for cost reduction of storage of *Mtb* biospecimens for future research in low income countries. In addition, the findings facilitated a clear understanding of the results of objectives 3 and 4.

Chapter 6. Chapter 6 describes the molecular epidemiology of RR/MDR-TB isolates of patients treated from 2011 to 2016 in the Southern region and 2015-2016 in the Northern region of Zimbabwe. Objectives 3 is answered in this chapter. We hypothesize that increased migration to and from the SADC by Zimbabweans had increased the circulation of *Mtb* strains that were previously not common in the country. Using spoligotyping and available secondary published data of RR/MDR-TB from the SADC countries, the chapter reports the strain lineage at molecular level and compares proportions of *Mtb* strains found in Zimbabwe with those from the SADC region. A map showing the percentage contribution of each of the *Mtb* genotypes was used to demonstrate the increased occurrence of the Beijing strain in Zimbabwe for both the Southern and Northern regions. Although there was no significance difference in mortality between the North and the South, the proportion of RR/MDR-TB patients who died was more from the Southern region.

Chapter 7. Chapter 7 describes the phylogeography of the Lineage 2 (L2) Beijing strain as it relates to migration between Zimbabwe and South Africa. This chapter

was intended to confirm whether the findings from Chapter 6 showing that the Lineage 2 Beijing strain had become predominant in some parts of Zimbabwe was due to migration. To increase the strengths of the study, we pooled whole genome sequencing results from a previous study in the Northern part of Zimbabwe with the study isolates. The lineage 2 genotypes in Zimbabwe were compared with those from South Africa to assess relatedness and resistance mutations. In addition, we showed the directionality of the Beijing strain transmission over time and estimated the time migration-related transmission could have started. This chapter provides the main result on migration and transmission of RR/MDR-TB between Zimbabwe and South Africa. My contribution in this chapter was design of the study, assistance with extraction of DNA and writing the paper. My co-authors assisted with the bioinformatics analysis, and design of the NEXTTRAIN software used to generate directionality outputs. Objective 4 is answered in this chapter.

Chapter 8. Chapter 8 provides the general conclusions and recommendations to Zimbabwe and the SADC region on the best practices to manage cross border transmission of RR/MDR-TB. Key recommendations on strengthening laboratory techniques and health system strengthening to be able to track MDR-TB patients as they move within the region and to ensure that TB patients have access to treatment and care irrespective of migration status.

References

1. Rushton K. The Records of Stannington Children's Sanatorium: Charting Half a Century of Tuberculosis Care. Soc Hist Med. 2016 Nov;29(4):829–39.

2. World Health Organization. Global tuberculosis report 2017. 2017.

3. World Health Organization. Global tuberculosis report 2019.pdf. Geneva, Switzerland; 2019. Report No.: Licence: CC BY-NC-SA 3.0 IG.

4. World Health Organization. Global tuberculosis control: WHO report 2011. Geneva: World Health Organization; 2011.

5. World Health Organization. Global tuberculosis report 2018. 2018.

6. Timire C, Sandy C, Kumar AMV, Ngwenya M, Murwira B, Takarinda KC, et al. Access to second-line drug susceptibility testing results among patients with Rifampicin resistant tuberculosis after introduction of the Hain Line Probe Assay in Southern provinces, Zimbabwe. Int J Infect Dis. 2019 Apr;81:236–43.

7. Coscolla M, Barry PM, Oeltmann JE, Koshinsky H, Shaw T, Cilnis M, et al. Genomic epidemiology of multidrug-resistant Mycobacterium tuberculosis during transcontinental spread. J Infect Dis. 2015;jiv025.

8. Cohen KA, Manson AL, Abeel T, Desjardins CA, Chapman SB, Hoffner S, et al. Extensive global movement of multidrug-resistant *M. tuberculosis* strains revealed by whole-genome analysis. Thorax. 2019 Sep;74(9):882–9.

9. Casali L, Crapa ME. Women, immigration, poverty and tuberculosis. Multidiscip Respir Med. 2010;5(6):1.

10. Garzelli C, Lari N, Cuccu B, Tortoli E, Rindi L. Impact of immigration on tuberculosis in a low-incidence area of Italy: a molecular epidemiological approach. Clin Microbiol Infect. 2010 Nov;16(11):1691–7.

11. Goldberg SV. Pooling tides: the convergence of migration, congregation, and tuberculosis. Int J Tuberc Lung Dis. 2005;9(5):475–475.

12. Flahaux M-L, De Haas H. African migration: trends, patterns, drivers. Comp Migr Stud. 2016 Dec;4(1):1.

13. Shimeles A. Migration Patterns, Trends and Policy Issues in Africa. African Development Bank Group; 2010 Dec p. 38. (Working paper series). Report No.: 119.

14. Lurie MN, Williams BG. Migration and health in Southern Africa: 100 years and still circulating. Health Psychol Behav Med Open Access J. 2014;2(1):34–40.

15. Crush J, Williams V, Peberdy S. Migration in southern Africa. Policy Anal Res Programme Glob Comm Int Migr [Internet]. 2005 [cited 2017 Feb 7];

16. Cain KP, Marano N, Kamene M, Sitienei J, Mukherjee S, Galev A, et al. The movement of multidrug-resistant tuberculosis across borders in East Africa needs a regional and global solution. PLoS Med. 2015;12(2):e1001791.

17. Yang C, Lu L, Warren JL, Wu J, Jiang Q, Zuo T, et al. Internal migration and transmission dynamics of tuberculosis in Shanghai, China: an epidemiological, spatial, genomic analysis. Lancet Infect Dis. 2018 Jul;18(7):788–95.

18. Basu S, Stuckler D, Gonsalves G, Lurie M. The production of consumption: addressing the impact of mineral mining on tuberculosis in southern Africa. Glob Health. 2009;5(1):11.

19. Zanamwe L, Devillard A, Zimbabwe National Statistics Agency, International Organization for Migration. Migration in Zimbabwe: a country profile 2009. Harare, Zimbabwe: Zimbabwe National Statistics Agency : International Organization for Migration; 2010.

20. Dodson B, Crush J. Migration Governance and Migrant Rights in the Southern African Development Community (SADC). :27.

21. Pareek M, Greenaway C, Noori T, Munoz J, Zenner D. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. BMC Med [Internet]. 2016 Dec [cited 2017 Jan 29];14(1).

22. Saleh DJ-EA. Globalization and the Spread of Multi-Drug Resistant Tuberculosis. 2015;7.

23. Walker TM, Lalor MK, Broda A, Saldana Ortega L, Morgan M, Parker L, et al. Assessment of Mycobacterium tuberculosis transmission in Oxfordshire, UK, 2007-12, with whole pathogen genome sequences: an observational study. Lancet Respir Med. 2014 Apr;2(4):285–92.

24. Moonan PK, Bayona M, Quitugua TN, Oppong J, Dunbar D, Jost KC, et al. Using GIS technology to identify areas of tuberculosis transmission and incidence. Int J Health Geogr. 2004;3(1):23.

25. Takarinda KC, Sandy C, Masuka N, Hazangwe P, Choto RC, Mutasa-Apollo T, et al. Factors Associated with Mortality among Patients on TB Treatment in the Southern Region of Zimbabwe, 2013. Tuberc Res Treat. 2017;2017.

26. Smith KLJ, Saini D, Bardarov S, Larsen M, Frothingham R, Gandhi NR, et al. Reduced Virulence of an Extensively Drug-Resistant Outbreak Strain of Mycobacterium tuberculosis in a Murine Model. Karakousis PC, editor. PLoS ONE. 2014 Apr 14;9(4):e94953.

27. Sagonda T, Mupfumi L, Manzou R, Makamure B, Tshabalala M, Gwanzura L, et al. Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resistant Tuberculosis Isolates in Zimbabwe. Tuberc Res Treat. 2014;2014:1–8.

28. Chihota V, Apers L, Mungofa S, Kasongo W, Nyoni IM, Tembwe R, et al. Predominance of a single genotype of Mycobacterium tuberculosis in regions of Southern Africa. Int J Tuberc Lung Dis. 2007;11(3):311–318.

29. Gushulak BD, Weekers J, MacPherson DW. Migrants and emerging public health issues in a globalized world: threats, risks and challenges, an evidence-based framework. Emerg Health Threats J. 2009;2(1):7091.

30. Dowdy DW, Golub JE, Chaisson RE, Saraceni V. Heterogeneity in tuberculosis transmission and the role of geographic hotspots in propagating epidemics. Proc Natl Acad Sci. 2012 Jun 12;109(24):9557–62.

Chapter 2

Migration and multidrug resistant tuberculosis: emerging public health challenge

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Migration and multidrug resistant tuberculosis: emerging public health challenge

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Abstract

Evidence of migration as a recognized method of spreading multidrug resistant tuberculosis (MDR-TB) remains limited. The only available evidence describes migration from high burden to low burden settings with minimal research outputs from high burden countries. This narrative literature review aimed to discuss available evidence on migration and transmission of MDR-TB and highlight knowledge gaps. The key findings were that the economically active age-group of 20 to 60 years, with more males than females were commonly migrating. Main drivers of migration from high to low MDR-TB burden countries were low socioeconomic factors, conflict and commerce. Unlike in migration between two high burden countries, mixing patterns of immigrants and local populations to low burden countries were minimal. The presence of a low mixing captive immigrant population plus available resources for research in low burden settings ensured that there were more studies unlike in high burden low resource settings. Available guidelines for the management of migration related MDR-TB were based on studies done in low burden countries. These may not be relevant in high burden countries where HIV and under nutrition are the major contributing factors to MDR-TB transmission. We conclude that despite lack of adequate evidence, there was probably significant MDR-TB transmission due to migration in high burden countries. The weak public health systems to allow for early diagnosis and treatment will make the prevention and control of migration related MDR-TB impossible in high burden countries. Strengthening national TB control programmes to improve access to MDR-TB care and implementation of collaborative research are an urgent priority.

Key words: Migration, Multidrug resistant TB, Transmission, Cross border, Migrant

2.1 Introduction

Multidrug resistance tuberculosis (MDR-TB), defined as resistance to isoniazid (INH) and rifampicin (RIF), has become an emerging global public health challenge with more than half a million people (558 000) notified in 2017 globally (1). In the Southern Africa Development Community (SADC) region, there were five (5) countries including Zimbabwe, categorised as high triple TB, MDR-TB and human immunodeficiency virus (HIV) burden countries (1). Drug resistant TB results from either spontaneous genetic mutation that confers resistance to anti-TB drugs or acquired resistance from inadequate treatment that results in genetic mutation (2,3). Transmission of drug resistant TB (primary) is largely due to inadequate programmatic factors like poor infection prevention and control, and erratic quality anti-TB medicines supply resulting in prolonged exposure of susceptible individuals, contact with an infectious person with cavitary disease and poor treatment adherence (3–5). Exposure to an infectious TB person may result in either chronic latent TB infection, progression to active TB disease or complete resolution of the TB infection. Poverty, malnutrition, and HIV infection promote the progression to TB disease post exposure and smoking and overcrowding, all common in low income countries, increase the risk of *Mycobacterium tuberculosis* (*Mtb*) transmission (6,7). The Zimbabwe 2016 drug resistance survey showed that 42/62 (68%) of the RR/MDR-TB were new cases, an indication that primary transmission may have been the major driver of DR TB (8).

The migration phenomenon started in the 16th century as a form of forced movement of people (9). As of mid-2017, the United Nations reported a total of 244 million international documented and undocumented migrants, with a net movement to the north from south. Majority of the immigrants were male, 51.9%, between the age-group 20 to 64 years (10). In the Southern hemisphere, this population has the highest

HIV prevalence and is more likely to be co-infected with TB (11). Early historical description of TB, the *"white plague"* in the period before Christ was in North Africa, India, China, the Americas, and Europe (12–14). In low burden countries, migration from the United Kingdom (UK) to Canada during the European industrial revolution is believed to have caused successful spread of TB into Canada around the 1900 period (14).

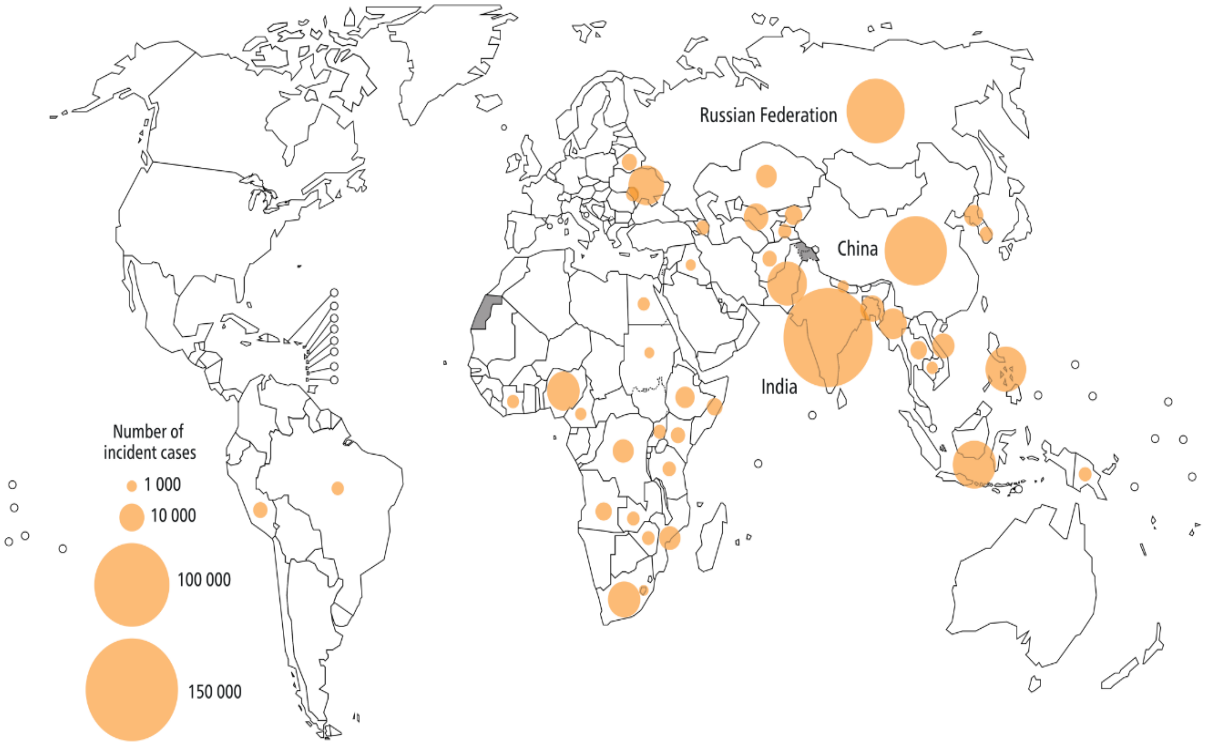

Figure 1. Estimated incidence of RR/MDR-TB, for countries with at least 1000 incident cases, 2016 (15)

Source: With permission from the WHO Global TB Programme and END TB

Current knowledge on the factors promoting occurrence of MDR-TB among immigrants have largely been from the World Health Organization's (WHO) European region where an estimated 78,000 MDR-TB new cases were being reported annually (16). Recommendations on the management of MDR-TB during migration processes were based on evidence from high income countries. These recommendations may not be relevant to low income countries where the national tuberculosis control

programmes (NTP) are poorly resourced (17). Understanding the mechanism of MDR-TB transmission related to migration, in high burden settings will assist in developing appropriate guidelines for the prevention and control of MDR-TB. This review aimed to describe the global epidemiology of migration-related MDR-TB especially in high MDR-TB burden countries.

2.2 Methodology

A narrative literature review was used to describe available evidence on migration and spread of RR/MDR-TB. For purposes of this review, migration was defined as both forced movement by war or social unrest and socioeconomic circumstances between countries and within the country. Published literature in peer reviewed journals, WHO database and international organization of migration (IOM) reports were accessed from PubMed, Biomed Central, Directory of Open Access Journals, SpringerOpen and Embase. The following search terms were used to search for published literature:

- Tuberculosis AND Resistant AND (migration OR immigration OR travel OR refugee OR cross border)
- Tuberculosis AND Resistant AND migrants AND (Prevention OR Control OR Treatment)
- Tuberculosis AND Resistant AND migrants AND (Prevention OR Control OR Treatment) AND (GeneXpert OR molecular OR solid OR liquid culture OR drug sensitivity testing)

There was no limitation of geographical setting and date of publication of the published literature. Only research and review papers that reported MDR-TB in humans, diagnosed using GeneXpert and other molecular techniques, solid or liquid culture and drug sensitivity testing (DST) were included in the study. Available references of

reviewed articles were also accessed and included to increase the coverage of the articles. All study designs were included. The outcomes of interest were prevalence of RR/MDR-TB among migrant and native populations, diagnostic methods used to characterise strain variability and MDR-TB treatment outcomes.

2.3 Results

A total of 668 studies were accessed and after filtering for free full text articles, there were 270 remaining studies. After filtering for studies involving human TB, 216 studies were available for review. Of these, twelve (12) were on drug resistant bacteria other than TB so they were excluded. An additional 118 studies reported migration and drug sensitive TB transmission. These were excluded except one review paper that described control of migration related TB in low incidence countries. Eighty-six papers were included in the narrative review.

2.3.1 Migration and RR/MDR-TB spread from high to low burden settings

The current global burden of MDR-TB shows that India, China and Russia contribute 50% of all MDR-TB cases (1). Out of the 155/194 WHO member countries reporting MDR-TB in 2017, the majority were from the Asian, Russian federation, Central and Southern Africa sub-continents (Figure 1) (15). The first anti-TB drug resistance report was in 1940 with the discovery of first anti-TB drug. This was followed by MDR-TB outbreaks in the United States of America (USA) around 1970 to 1990s (18). In response to the MDR-TB outbreaks, the WHO in 1994, started a twenty year global MDR-TB surveillance programme which showed that the increased prevalence levels of MDR-TB in Russia, Austria, Finland, Sweden, and the United Kingdom were due to immigration (19). The most intense intercountry migration related MDR-TB spread (20) was from the European region. Transcontinental spread of MDR-TB was most

intense between the Asian subcontinent and North America and between Europe and the African subcontinent (20,21). Two whole genome analysis (WGS) studies confirmed presence of extensive intercontinental spread of MDR-TB. One of the studies showed that about 17/24 (71%) of African, Asian and European countries contributed immigrants with MDR-TB (22). The other molecular study was used to demonstrate that despite the observed increase in the incidence of MDR-TB in Thailand and California, the cases remained within immigrants due to low social mixing and poverty (23).

All the studies used molecular studies to differentiate imported MDR-TB strains from local MDR-TB strains. Spread of MDR-TB from high to low burden countries was evident. However, because of the low mixing patterns and poverty among the immigrants, there was no demonstrable transmission between immigrants and natives. In addition, description by Pareek et al in a review of migration and drug sensitive TB, that the risk of TB among immigrants reduced to that of locals after 5 years of stay may suggest that there was minimal active transmission but largely re-activation of latent TB (24). The low mixing patterns and molecular epidemiological research capacity in receiving countries allowed the generation of evidence on spread of MDR-TB due to migration in Europe and North America.

2.3.2 Migration and Spread of RR/MDR-TB between High Burden Countries

Studies on intercountry spread of MDR-TB between high burden countries have been few. Intercountry transmission between high burden countries outside conflict situations was described in the SADC, West and East African regions(25–27). In Southern Africa, migration related to gold mine workforce in South African mines has been implicated. Mathema et al used IS6110-based restriction fragment length polymorphisms and spoligotyping analysis to describe the transmission in South

African gold mines. Cases from Mozambique and Lesotho were clustered and of different genotype compared to South African patients. The observed variationin genotypic patterns among the cases from Lesotho and Mozambique may suggest re-activation of latent TB rather infection in South African mines. In Cameroon, the authors used demographic and drug sensitivity tests (DST) to report patients from Equatorial Guinea who failed the standardized treatment regimen (26). Circumstances in Cameroon and the East Africa region were similar in terms of the drivers of migration, inadequate treatment services from countries of origin (27). Conflict situations have been rare in the SADC region, but severe socioeconomic factors have affected most of the countries except South Africa, Botswana and Namibia. A paper describing the prevention and control of MDR/XDR-TB in Southern Africa recommended adequate prevention measures in both South Africa and labour sending countries to interrupt transmission driven by mine workers (28). Presence of undetected TB at autopsy for about 40% of black miners in 1994 may suggest that propagation of TB and possibly drug resistant TB started before capacity to diagnose MDR-TB was available (29). Within the European region MDR-TB transmission using WGS and epidemiological links reported transmission from high burden countries to low burden countries, Germany and Austria (30)

Published workshop proceedings on addressing the threat of MDR-TB acknowledged that the under-reporting of MDR-TB from Africa was primarily due to inadequate diagnostic capacity (32). The authors used Lesotho as a case study, documented and undocumented mine workers were cited as the main drivers of the cross border MDR-TB transmission. Transmission was believed to be from mine workers to their unsuspecting immediate family members. Using epidemiological data notified to WHO, the Eastern, Central and Southern African (ECSA) regional TB prevention and

control framework confirmed the presence of increased migration in the region, with it, increasing the spread of TB(33). Saleh et al suggests that re-activation was the commonest cause of MDR-TB in high burden countries, due to the high levels of poverty, HIV infection and poor treatment success rates (32).

2.3.3 Internal migration and spread of DR-TB

Drivers of internal movement or intra-country migration, of people were mainly socio-political resulting in high unemployment and disparities in livelihoods (34). Intra-country migration-related TB and MDR-TB transmission has been reported in South Africa, Peru, Russia, Brazil and China (35–40). In Southern Africa, the spread of drug sensitive TB related to migration was described in the late 1800 period from urban mining to rural areas. During the early 1900 gold rush in South Africa, the working-class males contributed to the spread of TB from urban to rural settings (34). The overcrowded living conditions in the South African mines were conducive for TB transmission (41). Compared to intercountry migration, MDR-TB cases in intra-country migration were more likely to be new patients except for the study from China (42). This observation may indicate active transmission in high MDR-TB burden countries with intra-country migration, when compared to MDR-TB reported from high income countries. Studies from South Africa showed differential distribution of MDR-TB strains at molecular level across provinces, that is, the Beijing MDR-TB strains were more prevalent in the Western- and Eastern Cape and LAM 9 strain was more prevalent in Kwazulu-Natal and Gauteng provinces (37). Studies showing localised hotspot transmission of TB and MDR-TB may be an indication that short exposure periods to an infected person could be a significant risk factor for MDR-TB transmission (43).

2.3.4 Characteristics of immigrants spreading MDR-TB

Demographic characteristics of MDR-TB associated with migration were similar across the migration patterns. Immigrants were more likely to be male (64%), of younger economically age-group, 15-44 years (92%), with a positive history of traveling back to country of origin two years prior to the MDR-TB disease and likely to be co-infected with HIV (20,44,45). Where immigrant screening services were available, in Europe, 50% of the immigrants did not show signs and symptoms of TB on arrival. Common regions of immigrant origin were Asia, South America, Eastern Europe and Africa. Conditions of overcrowding, reduced access to health care services and inadequate nutrition were associated with increased risk of MDR-TB among migrants (46). Migration follow up studies on both MDR-TB and drug sensitive TB showed that the increased risk of MDR-TB among foreign born populations declined after 5 years of stay in low income countries. In addition, there was minimal evidence on the transmission of MDR-TB between migrants and natives (24). Unlike in the spread of MDR-TB between high to low burden countries, where low socioeconomic factors have been the major causes of migration, conflict situations were the major reasons for migration between high MDR-TB burden countries (47).

2.4 Discussion

2.4.1 Transmission of MDR-TB and Migration

The use of molecular epidemiology has been used to describe the contribution of migration to the spread of MDR-TB (48). In all the studies, there was minimal to no evidence of active transmission, but demonstrated the presence of foreign born persons with MDR-TB especially where migration involved movement from high to low

burden RR/MDR-TB settings. The hypothesis that migration promotes spread of MDR-TB has not been adequately answered and is still an area of research need. The observed limited transmission of MDR-TB cases between immigrants and natives may reflect the strength of TB programmes in high income countries. In high MDR-TB burden settings, active TB transmission from migration maybe possible, but difficult to demonstrate because of limited research infrastructure capacity. The WHO estimates that only 160,684 (28.8%) RR/MDR-TB cases were diagnosed and reported in 2017 out of the possible estimated 558,000 cases [1]. Majority of these missed cases were from low income countries.

Key differences in migration patterns between the low to high income countries and between low income countries may pose challenges in development of appropriate interventions against migration related MDR-TB in high burden countries. First, the fluidity in mixing patterns could mean that transmission is bi-directional further propagating the MDR-TB epidemic. Secondly, absence of a quantified risk of baseline MDR-TB transmission in high burden countries made it difficult to quantify the burden of migration related MDR-TB. Third, the high MDR-TB mortality in high burden countries in the absence of research evidence on treatment outcomes in the migrant population further makes programming of interventions challenging (49). Building capacity for MDR-TB research in high migration and MDR-TB burden settings is an urgent requirement.

2.4.2 Challenges in demonstrating migration and transmission of MDR-TB

Confirmation of migration associated transmission of MDR-TB in high burden countries has not been possible due to inadequate laboratory capacity in most African countries apart from South Africa (32,37). Molecular techniques in Europe and the United States of America demonstrated distinct Mtb strain variability between

immigrants and natives. Dowdy et al showed that adequate resources and programmatic response to MDR-TB was key to the reduction in the burden of MDR-TB in the USA (50). Inadequate laboratory capacity in low income countries had affected MDR-TB case finding and reporting resulting in apparent low incidence of MDR-TB (51). The potential likelihood of re-infection in high burden settings like Africa may also make determination of active transmission a challenge, making use of WGS the only most appropriate tool for MDR-TB diagnosis. Available literature from high income countries have largely demonstrated the high incidence of MDR-TB and TB cases among foreign born nationals rather than demonstrating transmission (52,53). Few studies have confirmed MDR-TB transmission during air travel where the likelihood of successful transmission was associated with infectiousness of the index case, proximity of susceptible individuals to the index case and duration of exposure (54,55).

2.4.3 Factors Promoting MDR-TB Transmission in Migrants

Risk factors for MDR-TB transmission under migration settings differ with the primary cause of migration. Factors that predict successful transmission were prolonged duration of exposure, infectiousness of the infected person, over crowdedness, under nutrition of exposed persons and low socioeconomic status (56). Under conflict situations, the underlying malnutrition, overcrowding in refugee camps and inadequate access to health facilities have been reported as the commonest [39]. Similar environmental exposures could promote MDR-TB transmission even within countries experiencing internal displacement and low socioeconomic conditions. In Southern Africa, the high mining activities were associated with overcrowded living conditions in hostels and dust environment from the mining activities (29). Besides a few reports from Lesotho and South Africa, on the contribution of mining activities to the burden

of MDR-TB in Southern Africa, there has not been evidence on what factors could promote MDR-TB transmission during cross border movement and internal migration(41,57). Most of the studies relied on surveillance data of patients seeking treatment for MDR-TB. The development of effective and appropriate MDR-TB interventions in high burden settings will rely on the availability of local research evidence. In high income countries, risk reduction after 5 years of arrival of immigrants, and comparable treatment outcomes (Pareek et al) suggest that the absence of environmental factors promoting MDR-TB transmission after migration in high income countries had an impact of reducing MDR-TB transmission.

2.5 Limitations of the study

This study used full text free articles only to discuss the epidemiology of DR-TB and migration. The researcher did not have financial resources to access articles on sale. This may have introduced some information bias with the results resulting in either over or under-estimating the impact of migration on the epidemiology of DR-TB. However, this bias may not have influenced the conclusion of our results given the paucity of data from low income and high DR-TB burden countries on migration related DR-TB epidemiology.

2.6 Conclusions

Physical spread of infected individuals migrating from high burden to low burden countries was evident. The low mixing patterns between immigrants and natives in low burden settings limited the MDR-TB infection to immigrants, with the resulting effect only on increased notification rather than propagated transmission. In high MDR-TB burden settings, the evidence on MDR-TB spread due to migration was limited to none. This was due to a combination of inadequate laboratory and research capacity. This still remains an area of research need.

Trade, commerce, forced migration from conflicts, and low socioeconomic factors were the main drivers of migration. Continued labour movement from low to high income countries and within the African region means that the threat of MDR-TB transmission will remain a reality. In high burden settings, the additional high HIV prevalence, low socioeconomic factors and inadequate health infrastructure to provide access to universal health care maybe promoting transmission of MDR-TB during and after migration activities. The poor access to health care services may also be potentiating the development of drug resistant TB from delayed identification of suboptimal treatment regimens. With continued inadequate funding for public health programmes, the threat of MDR-TB will continue to grow.

Despite the absence of concrete evidence on the link between migration and spread of MDR-TB, several programmatic implications to reduce transmission can be recommended. Key among the recommendations are those not amenable to health interventions and require non-health sector contribution, for example, promotion of adequate nutrition, reducing overcrowding and minimizing poverty [15]. Universal

access to quality health services will remain challenging in high MDR-TB burden countries where health care financing is inadequate (24,58).

2.7 Public health implications of MDR-TB spread and Migration

Absence of research evidence pose several challenges to the management of MDR-TB related to migration, especially in high burden settings. First, the development of comprehensive global guidelines for the control of MDR-TB under all forms of migration remains challenging. The WHO International Health Regulations (IHR) on MDR-TB and air travel recommends immediate notification of MDR-TB cases to WHO based on evidence from high income countries (59). There are no WHO guidelines on the prevention and control of MDR-TB under migration settings. Secondly, the inadequate universal access to care and treatment in most high MDR-TB burden countries, more so during migration would continue to promote transmission under programmatic conditions. Early diagnosis and treatment among immigrants were identified as key interventions to reduce MDR-TB transmission during and after migration as shown by high treatment success rates of about 70% among immigrants accessing MDR-TB treatment services in high income countries (60).

Thirdly, the practicability of using highly sensitive MDR-TB screening techniques and symptom screening in migration between high MDR-TB burden countries and intra-country migration is an area of research need. Screening programmes during migration have been recommended in low MDR-TB incidence countries, using both symptom, chest x-rays, Mantoux and interferon gamma release assays (IGRAs). Whilst the economic value of the screening programmes was non-conclusive, screening increased awareness and those identified through screening were more likely to seek care and treatment as a condition to successfully migrate (61,62). The WHO recommends the symptom screening tool in high burden countries, in TB/HIV

care settings and community targeted TB case finding (63). The justification to use symptom screening in high MDR-TB burden countries was affordability. The low sensitivity of the symptom screening method especially in settings with high HIV prevalence makes it less appropriate in high MDR-TB burden countries (64,65).

Fourth, the practicability of ensuring universal access to diagnosis and treatment in the presence of inadequate local funding in high MDR-TB burden countries remains a challenge. The low treatment enrolment of notified MDR-TB of 25% associated with low successful treatment of 52% in 2017 in the WHO regions was an indication of poor access to early diagnosis and treatment. Treatment outcome studies from low MDR-TB burden countries have demonstrated that tailor-making regimens according to DST patterns improves treatment outcome results although the differences were not statistically significant. In high burden MDR-TB countries, a standardised treatment regimen was used mainly due to the high number of cases that made individualised regimens logistically impossible. As a result, transmission of MDR-TB related to migration in high burden settings may continue for the next several years until funding to improve early diagnosis and treatment is made available.

Lastly, estimating the true magnitude of the contribution of migration to continued MDR-TB transmission in low income countries is an urgent research and programmatic need especially from regions with increased free movement of people. Implementation of migration studies and MDR-TB transmission in high burden countries will provide more concrete evidence on the contribution of migration to international spread of MDR-TB as well as assist the strengthening of national TB programmes. With the introduction of the new short term and individualized MDR-TB treatment regimens, these studies become more urgent. The ascertainment of *Mtb* strain types and transmission has not been widely available from high burden

countries except for South Africa (66). Few molecular studies in Zimbabwe, Kenya and Zambia have provided insights into predominant strains circulating in the SADC region (67,68). This evidence will facilitate future studies on migration and MDR-TB. Availing newly introduced whole genome sequencing (WGS) technologies in high burden countries to diagnose TB could improve the knowledge of MDR-TB transmission in migration settings at local, regional and global.

2. 8. Key Recommendations

Studies from high income countries showed that there was risk reduction over time and low to minimal MDR-TB transmission between natives and immigrants. These observations may suggest several important epidemiological factors amenable to interventions (69,70). Firstly, low to minimal transmission between natives and immigrants could suggest that there is low mixing between natives and immigrants. Screening and early treatment of MDR-TB among immigrants maybe adequate to control transmission of MDR-TB under migration settings in high income countries. In high burden countries, there is need to validate the available screening models and implement the most appropriate screening methods. Screening of immigrants in high burden countries may also assist in creating awareness on the need to seek early care and treatment. Because of the high mixing patterns between immigrants and natives in high burden countries, there is need to prioritise universal access to early and quality diagnosis, care and treatment of MDR-TB. The improvement in MDR-TB diagnostic capacity could allow the generation of evidence on the contribution of migration to transmission of MDR-TB in high burden settings.

Secondly, the reduced risk of MDR-TB among immigrants living in high income countries may suggest that even within the immigrants, transmission was minimal due

to improved socioeconomic factors in receiving countries. Therefore, improving living conditions during and after migration among immigrants have an effect of reducing re-activation of MDR-TB. Third, studies on social determinants of health among immigrants from low income countries living in high income countries are recommended to understand transmission dynamics of MDR-TB. Fourth, the comparable MDR-TB treatment outcomes between immigrants and natives in high-income countries could mean that migration per se may not be a threat to MDR-TB transmission, provided there are strong health systems for early case detection and treatment. We recommend strengthening of national TB programmes (NTP) including other social services sector ministries that promote good shelter, nutrition and physical security during and after migration. This will ensure country specific capacity building towards improvement of access to health care services for all people irrespective of migration status. Health specific recommendations include the universal access to health care services for immigrants, strengthening of laboratory capacity, access to early treatment and care and supply chain management for medicines and other consumables.

Fifth, key recommendation is to build capacity for MDR-TB operational research in high burden countries. As a minimum, the countries must improve MDR-TB surveillance systems and generate data for use in future research on social determinants MDR-TB among migrants. Sixth, NTP from high burden countries must consider introducing whole genome sequencing (WGS) for MDR-TB diagnosis, drug sensitivity testing and outbreak investigation. This would contribute to towards effective control of MDR-TB, especially as the clinical management of the disease is moving towards individualised regimen. Seventh, high burden countries could collaborate as regions to implement research activities using WGS technologies,

following the model of the European Surveillance system for MDR-TB cross border prevention and control (4).

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References

1. World Health Organization. Global tuberculosis report 2018. 2018.

2. Odone A, Calderon R, Becerra MC, Zhang Z, Contreras CC, Yataco R, et al. Acquired and Transmitted Multidrug Resistant Tuberculosis: The Role of Social Determinants. Lee JW, editor. PLOS ONE. 2016 Jan 14;11(1):e0146642.

3. Leung ECC, Leung CC, Kam KM, Yew WW, Chang KC, Leung WM, et al. Transmission of multidrug-resistant and extensively drug-resistant tuberculosis in a metropolitan city. Eur Respir J. 2013 Apr;41(4):901–8.

4. Barroso EC, Mota RMS, Santos RO, Sousa ALO, Barroso JB, Rodrigues JLN. Risk factors for acquired multidrug-resistant tuberculosis. J Pneumol. 2003;29(2):89–97.

5. Trauner A, Borrell S, Reither K, Gagneux S. Evolution of Drug Resistance in Tuberculosis: Recent Progress and Implications for Diagnosis and Therapy. Drugs. 2014 Jul;74(10):1063–72.

6. Andrews JR, Shah NS, Weissman D, Moll AP, Friedland G, Gandhi NR. Predictors of Multidrug- and Extensively Drug-Resistant Tuberculosis in a High HIV Prevalence Community. Tyagi AK, editor. PLoS ONE. 2010 Dec 29;5(12):e15735.

7. Gobena D, Ameya G, Haile K, Abreha G, Worku Y, Debela T. Predictor of multidrug resistant tuberculosis in southwestern part of Ethiopia: a case control study. Ann Clin Microbiol Antimicrob [Internet]. 2018 Dec [cited 2019 Mar 9];17(1).

8. Timire C, Metcalfe JZ, Chirenda J, Scholten JN, Manyame-Murwira B, Ngwenya M, et al. Prevalence of drug-resistant tuberculosis in Zimbabwe: a health facility-based cross-sectional survey. Int J Infect Dis. 2019 Jul;S1201971219303066.

9. Hendriks M. The happiness of international migrants: A review of research findings. Migr Stud. 2015;3(3):343–369.

10. United Nations. World Population prospects: Key findings and advance tables. Department of Economic and Social Affairs Population Division; 2017.

11. Long K. When refugees stopped being migrants: Movement, labour and humanitarian protection. Migr Stud. 2013 Mar 1;1(1):4–26.

12. Frith J. History of Tuberculosis. Part 1 - Phthisis, consumption and the White Plague. 2014;22(2):7.

13. Daniel TM. The history of tuberculosis. Respir Med. 2006 Nov;100(11):1862–70.

14. Wilton P. “TB voyages” into High Arctic gave MDs a look at a culture in transition. CAN MED ASSOC J. 1993;148(9):2.

15. World Health Organization. Global tuberculosis report 2017. 2017.

16. Jakab Z, Acosta C, Kluge H, Dara M. Consolidated Action Plan to Prevent and Combat Multidrug- and Extensively Drug-resistant Tuberculosis in the WHO European Region 2011-2015: Cost-effectiveness analysis. Tuberc Edinb. 2015 Jun;95(Suppl 1):S212-6.

17. Gushulak B, Weekers J, MacPherson D. Migrants in a globalized world ? health threats, risks and challenges: an evidence-based framework. Emerg Health Threats J [Internet]. 2008 [cited 2017 Jun 19];2.

18. Keshavjee S, Farmer PE. Tuberculosis, Drug Resistance, and the History of Modern Medicine. N Engl J Med. 2012 Sep 6;367(10):931–6.

19. Zignol M, Dean AS, Falzon D, van Gemert W, Wright A, van Deun A, et al. Twenty years of global surveillance of antituberculosis-drug resistance. N Engl J Med. 2016;375(11):1081–1089.

20. Faustini A. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax. 2006 Feb 1;61(2):158–63.

21. Metcalfe JZ, Kim EY, Lin S-YG, Cattamanchi A, Oh P, Flood J, et al. Determinants of Multidrug-Resistant Tuberculosis Clusters, California, USA, 2004–2007. Emerg Infect Dis. 2010 Sep;16(9):1403–9.

22. Cohen KA, Manson AL, Abeel T, Desjardins CA, Chapman SB, Hoffner S, et al. Extensive global movement of multidrug-resistant *M. tuberculosis* strains revealed by whole-genome analysis. Thorax. 2019 Sep;74(9):882–9.

23. Coscolla M, Barry PM, Oeltmann JE, Koshinsky H, Shaw T, Cilnis M, et al. Genomic epidemiology of multidrug-resistant Mycobacterium tuberculosis during transcontinental spread. J Infect Dis. 2015;jiv025.

24. Pareek M, Greenaway C, Noori T, Munoz J, Zenner D. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. BMC Med [Internet]. 2016 Dec [cited 2017 Jan 29];14(1).

25. Mathema B, Lewis JJ, Connors J, Chihota VN, Shashkina E, van der Meulen M, et al. Molecular Epidemiology of *Mycobacterium tuberculosis* among South African Gold Miners. Ann Am Thorac Soc. 2015 Jan;12(1):12–20.

26. Noeske J, Foe J-LA, Kuaban C. Cameroon's MDR-TB treatment programme jeopardised by cross-border migration. Eur Respir J. 2016 Feb;47(2):684–6.

27. Cain KP, Marano N, Kamene M, Sitienei J, Mukherjee S, Galev A, et al. The movement of multidrug-resistant tuberculosis across borders in East Africa needs a regional and global solution. PLoS Med. 2015;12(2):e1001791.

28. Grobusch MP. Drug-resistant and extensively drug-resistant tuberculosis in southern Africa: Curr Opin Pulm Med. 2010 Feb;1.

29. Stuckler D, Steele S, Lurie M, Basu S. Introduction: “dying for gold”: the effects of mineral miningon HIV, tuberculosis, silicosis, and occupational diseases in southern Africa. Int J Health Serv Plan Adm Eval. 2013;43(4):639–49.

30. Fiebig L, Kohl TA, Popovici O, Mühlenfeld M, Indra A, Homorodean D, et al. A joint cross-border investigation of a cluster of multidrug-resistant tuberculosis in Austria, Romania and Germany in 2014 using classic, genotyping and whole genome sequencing methods: lessons learnt. Eurosurveillance. 2017;22(2).

31. Hollo V, Kotila SM, Ködmön C, Zucs P, van der Werf MJ. The effect of migration within the European Union/European Economic Area on the distribution of tuberculosis, 2007 to 2013. Eurosurveillance [Internet]. 2016 Mar 24 [cited 2018 Aug 18];21(12).

32. Giffin R, Robinson S. Addressing the Threat of Drug-Resistant Tuberculosis: A Realistic Assessment of the Challenge: Workshop Summary. IOM Inst Med DC Natl Acad Press Copyr © Natl Acad Sci Rights Reserv. 2009;253.

33. East, Central & Southern Africa Health Community. A Strategic Framework for Cross-Border and Regional Programming in TB Prevention and Control for ECSA Region 2015. TB Care I; 2015.

34. Kimbrough W, Saliba V, Dahab M, Haskew C, Checchi F. The burden of tuberculosis in crisis-affected populations: a systematic review. Lancet Infect Dis. 2012 Dec;12(12):950–65.

35. van Halsema CL, Fielding KL, Chihota VN, Lewis JJ, Churchyard GJ, Grant AD. Trends in drug-resistant tuberculosis in a gold-mining workforce in South Africa, 2002–2008. Int J Tuberc Lung Dis. 2012 Jul 1;16(7):967–73.

36. Droznin M, Johnson A, Johnson AM. Multidrug resistant tuberculosis in prisons located in former Soviet countries: A systematic review. Manganelli R, editor. PLOS ONE. 2017 Mar 23;12(3):e0174373.

37. Wang W, Wang J, Zhao Q, Darling ND, Yu M, Zhou B, et al. Contribution of rural-to-urban migration in the prevalence of drug resistant tuberculosis in China. Eur J Clin Microbiol Infect Dis. 2011 Apr;30(4):581–6.

38. Zelner JL, Murray MB, Becerra MC, Galea J, Lecca L, Calderon R, et al. Identifying Hotspots of Multidrug-Resistant Tuberculosis Transmission Using Spatial and Molecular Genetic Data. J Infect Dis. 2016 Jan 15;213(2):287–94.

39. Ershova JV, Volchenkov GV, Kaminski DA, Somova TR, Kuznetsova TA, Kaunetis NV, et al. Epidemiology of Primary Multidrug-Resistant Tuberculosis, Vladimir Region, Russia. Emerg Infect Dis. 2015 Nov;21(11):2048–51.

40. Al-Hajoj S, Varghese B, Shoukri MM, Al-Omari R, Al-Herbwai M, AlRabiah F, et al. Epidemiology of Antituberculosis Drug Resistance in Saudi Arabia: Findings of the First National Survey. Antimicrob Agents Chemother. 2013 May;57(5):2161–6.

41. Dantas NGT, Suffys PN, Carvalho W da S, Gomes HM, de Almeida IN, de Assis LJ, et al. Genetic diversity and molecular epidemiology of multidrug-resistant

Mycobacterium tuberculosis in Minas Gerais State, Brazil. BMC Infect Dis [Internet]. 2015 Dec [cited 2016 Jun 12];15(1).

42. Lurie MN, Williams BG. Migration and health in Southern Africa: 100 years and still circulating. Health Psychol Behav Med Open Access J. 2014;2(1):34–40.

43. Ding P, Li X, Jia Z, Lu Z. Multidrug-resistant tuberculosis (MDR-TB) disease burden in China: a systematic review and spatio-temporal analysis. BMC Infect Dis [Internet]. 2017 Dec [cited 2017 Jan 31];17(1).

44. Yablonskii PK, Vizel AA, Galkin VB, Shulgina MV. Tuberculosis in Russia. Its History and Its Status Today. Am J Respir Crit Care Med. 2015 Feb 15;191(4):372–6.

45. Anderson LF, Tamne S, Brown T, Watson JP, Mullarkey C, Zenner D, et al. Transmission of multidrug-resistant tuberculosis in the UK: a cross-sectional molecular and epidemiological study of clustering and contact tracing. Lancet Infect Dis. 2014;14(5):406–415.

46. Dierberg KL, Dorjee K, Salvo F, Cronin WA, Boddy J, Cirillo D, et al. Improved detection of tuberculosis and multidrug-resistant tuberculosis among tibetan refugees, India. Emerg Infect Dis. 2016;22(3):463.

47. Cohen T, Jenkins HE, Lu C, McLaughlin M, Floyd K, Zignol M. On the spread and control of MDR-TB epidemics: An examination of trends in anti-tuberculosis drug resistance surveillance data. Drug Resist Updat. 2014 Oct;17(4–6):105–23.

48. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of

multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med. 2017 Apr;5(4):291–360.

49. Drobniewski F, Balabanova Y, Nikolayevsky V, Ruddy M, Kuznetzov S, Zakharova S, et al. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. JAMA. 8;293(22):2726-31.; 2005.

50. Dowdy DW, Theron G, Tornheim JA, Kendall EA. Drug-resistant tuberculosis in 2017: at a crossroads. Lancet Respir Med. 2017 Apr;5(4):241–2.

51. Garzelli C, Rindi L. Molecular epidemiological approaches to study the epidemiology of tuberculosis in low-incidence settings receiving immigrants. Infect Genet Evol. 2010 Jun;12(4):610–8.

52. Garzelli C, Lari N, Cuccu B, Tortoli E, Rindi L. Impact of immigration on tuberculosis in a low-incidence area of Italy: a molecular epidemiological approach. Clin Microbiol Infect. 2010 Nov;16(11):1691–7.

53. Sandgren A, Sane Schepisi M, Sotgiu G, Huitric E, Migliori GB, Manissero D, et al. Tuberculosis transmission between foreign- and native-born populations in the EU/EEA: a systematic review. Eur Respir J. 2014 Apr 1;43(4):1159–71.

54. Marienau K, Burgess G, Cramer E, Averhoff F, Buff A, Russell M, et al. Tuberculosis investigations associated with air travel: US Centres for Disease Control and Prevention, January 2007 June 2008. Travel Med Infect Dis. 2010 Mar 11;8(2):104–12.

55. Kenyon TA, Valway SE, Ihle WW, Onorato IM, Castro KG. Transmission of multidrug-resistant Mycobacterium tuberculosis during a long airplane flight. N Engl J Med. 1996;334(15):933–938.

56. Kapwata T, Morris N, Campbell A, Mthiyane T, Mpangase P, Nelson KN, et al. Spatial distribution of extensively drug-resistant tuberculosis (XDR TB) patients in KwaZulu-Natal, South Africa. Nicol MP, editor. PLOS ONE. 2017 Oct 13;12(10):e0181797.

57. Satti H, Seung K, Keshavjee S, Furin J. Extensively Drug-Resistant Tuberculosis, Lesotho. Emerg Infect Dis. 2008 Jun;14(6):992–3.

58. Helbling P, Altpeter E, Egger J, Zellweger J. Treatment outcomes of multidrug-resistant tuberculosis in Switzerland. Swiss Med Wkly [Internet]. 2014 Dec 4 [cited 2019 Feb 14];

59. Organization WH. Tuberculosis and air travel: guidelines for prevention and control [Internet]. World Health Organization; 2008 [cited 2017 Mar 19].

60. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment Outcomes of Multidrug-Resistant Tuberculosis: A Systematic Review and Meta-Analysis. Pai M, editor. PLoS ONE. 2009 Sep 9;4(9):e6914.

61. Lalvani A, Pareek M. Immigrant screening for TB: a missed opportunity to improve TB control in the United Kingdom. Pathog Glob Health. 2012 Mar;106(1):5–7.

62. Arnesen TM. Economic models of LTBI screening in migrants: need for harmonization of approach. Int J Tuberc Lung Dis. 2017;21(9):954–954.

63. Mor Z, Weinstein O, Tischler-Aurkin D, Leventhal A, Alon Y, Grotto I. The yield of tuberculosis screening of undocumented migrants from the Horn of Africa based on chest radiography. Isr Med Assoc J IMAJ. 2015 Jan;17(1):11–3.

64. Bloss E, Makombe R, Kip E, Smit M, Chirenda J, Gammino VM, et al. Lessons learned during tuberculosis screening in public medical clinics in Francistown,

Botswana [Notes from the field]. Int J Tuberc Lung Dis. 2012 Aug 1;16(8):1030–2.

65. Zhang C, Ruan Y, Cheng J, Zhao F, Xia Y, Zhang H, et al. Comparing yield and relative costs of WHO TB screening algorithms in selected risk groups among people aged 65 years and over in China, 2013. Gao L, editor. PLOS ONE. 2017 Jun 8;12(6):e0176581.

66. Warren RM. Implementation of new tools for multidrug-resistant tuberculosis detection and control. Int J Mycobacteriology. 2016;5:S67.

67. Githui WA, Jordaan AM, Juma ES, Kinyanjui P, Karimi FG, Kimwomi J, et al. Identification of MDR-TB Beijing/W and other Mycobacterium tuberculosis genotypes in Nairobi, Kenya. Int J Tuberc Lung Dis. 2004;8(3):352–360.

68. Chihota V, Apers L, Mungofa S, Kasongo W, Nyoni IM, Tembwe R, et al. Predominance of a single genotype of Mycobacterium tuberculosis in regions of Southern Africa. Int J Tuberc Lung Dis. 2007;11(3):311–318.

69. Hersi A, Elwood K, Cowie R, Kunimoto D, Long R. Multidrug-Resistant Tuberculosis in Alberta and British Columbia, 1989 to 1998. Can Respir J. 1999;6(2):155–60.

70. Stoffels K, Allix-Béguec C, Groenen G, Wanlin M, Berkvens D, Mathys V, et al. From Multidrug- to Extensively Drug-Resistant Tuberculosis: Upward Trends as Seen from a 15-Year Nationwide Study. Herrmann JL, editor. PLoS ONE. 2013 May 9;8(5):e63128.

Annex 1a. List of key references used
Section 2.3.1 Global distribution, spread from high burden to low burden

Reference	Authors	Title	Key findings
17	Salmaan Keshavjee and Paul E. Farmer	Tuberculosis, Drug Resistance, and the History of Modern Medicine	• The prevalence of DR-TB started early at the discovery of anti-TB medicines • Outbreaks of MDR-TB common among HIV positive • Evidence prompted the commissioning of a global MDR-TB surveillance programme
1 and 15	WHO	2017 and 2018 WHO global TB reports	• MDR-TB more prevalent from Asian, Russian federation, Central and Southern Africa sub-continents
19	Zignol M. Dean AS. Falzon D et al	Twenty years of global surveillance of antituberculosis drug resistance, N Eng J Med, 2016 Report of a 20 year global surveillance on MDR-TB	• Describing MDRTB trends was difficult in Africa and Asian countries despite these contributing more MDR-TB cases in countries receiving immigrants • Increased MDR-TB cases in European and a decrease in the USA, Australia and some parts of Europe • Reports of DR-TB reported soon after the discovery of anti-TB medicines • Incapacity of the Africa and Asian sub continents to diagnose MDR-TB made estimation of the disease from these continents difficult
20	A Faustini, A J Hall, C A Perucci	Risk factors for multidrug resistant tuberculosis in Europe: a systematic review	• Described risk factors for MDRTB • Confirmed foreign born persons were more likely to have MDR-TB

			• Young age, less than 65 years and HIV positive were more likely to have MDR-TB • According to the UN report on international migration, this age group was the commonest immigrants • Did not show significant cross transmission between immigrants and natives • Migration was from Sub Saharan Africa and Eastern Europe
21	John Z. Metcalfe, Elizabeth Y. Kim, S.-Y et al	Determinants of Multidrug-Resistant Tuberculosis Clusters, California, USA, 2004–2007 Population based cohort study from January 1, 2004, through December 31, 2007 in California	• 92% of the MDRTB cases were among the foreign born persons from Mexico, Philippines, the People's Republic of China, Vietnam, Thailand and Lao People's Democratic Republic Cases were more likely to be young and HIV positive
22	Keira A Cohen. Abigail L Manson. and Thomas Abeel,et al	Extensive global movement of multidrug-resistant M. tuberculosis strains revealed by whole genome analysis Phylogeographic study using samples from different countries	• Global spread of MDR-TB through migration • Utility of whole genome sequencing in investigating MDR-TB outbreaks and phylogeographic studies
23	Mireia Coscolla. Pennan M.Barry. John E.Oeltmann. et al	Genomic Epidemiology of Multidrug-Resistant Mycobacterium tuberculosis During Transcontinental Spread	• Confirmed that MDR-TB remained largely within the immigrant population • No active transmission
24	Manish Pareek. Christina	The impact of migration on tuberculosis	• Review of drug sensitive TB and migration which made specific

	Greenaway. Teymur Noori. et al	epidemiology and control in high-income countries: a review	recommendations for the control of TB in migration settings • Confirmed low mixing patterns between immigrants and natives

Annex 1b. List of key references used

Section 2.3.2 Spread of MDR-TB between High Burden Countries

Reference	Authors	Title	Key findings and methods
25	Steve Olson. Yeonwoo Lebovitz, and Anne Claiborne Institute of Medicine and the Academy of Science of South Africa	The Emerging Threat of Drug-Resistant Tuberculosis in Southern Africa Global and Local Challenges and Solutions SUMMARY OF A JOINT WORKSHOP	• Used epidemiological studies • Inadequate diagnostic capacity in most Southern African countries • Cross border mine workers contributing to spread of MDR-TB
26	East, Central & Southern Africa Health Community	Strategic Framework for Cross-Border and Regional Programming in Tuberculosis (TB) Prevention and Control for East, Central and Southern Africa Health Community (ECSA-HC) Region	• Describe the burden of Tb and MDR-TB associated with migration in the COMESA region, particularly Southern Africa region • Used epidemiological data
27	Kevin P. Cain. Nina Marano. Maureen Kamene. et al	The Movement of Multidrug-Resistant Tuberculosis across Borders in East Africa Needs a Regional and Global Solution	• Epidemiological description of MDR-TB cases from Somalia seeking treatment in Kenya • Described the need for regional and global response to migration related MDR-TB spread
28	Martin Peter Grobuscha	Drug-resistant and extensively drug-	• Used epidemiological evidence of history of

		resistant tuberculosis in southern Africa	DR-TB and XDRTB among mine workers from South Africa and the SADC region.
29	David Stuckler, Sarah Steele, Mark Lurie, and Sanjay Basu	INTRODUCTION: 'DYING FOR GOLD': THE EFFECTS OF MINERAL MININGON HIV, TUBERCULOSIS, SILICOSIS, AND OCCUPATIONAL DISEASES IN SOUTHERN AFRICA	• Secondary autopsy data from of former mine workers who had TB at autopsy. • There was no confirmation of DR-TB and the authors hypothesized that potential for DR-TB cases was high
30	Euro Surveill. 2017 Jan 12; 22(2): 30439.	A joint cross-border investigation of a cluster of multidrug-resistant tuberculosis in Austria, Romania and Germany in 2014 using classic, genotyping and whole genome sequencing methods: lessons learnt	• Molecular and epidemiological methods to confirm MDR-TB transmission and migration
31	Vahur Hollo. Saara Magdalena Kotila. Csaba Ködmön. et al	The effect of migration within the European Union/European Economic Area on the distribution of tuberculosis, 2007 to 2013	• Analysed routinely collected surveillance data of drug sensitive TB • Confirmed presence of regional spread of TB

Chapter 3

Prevalence of drug-resistant tuberculosis in Zimbabwe: a health facility based cross sectional survey

Collins Timire, [1,2] John Z Metcalfe, [3] Joconiah Chirenda, [4] Jerod N Scholten,[5] Barbara Manyame-Murwira,[2] Mkhokheli Ngwenya,[6] Ronnie Matambo[7], Kelvin Charambira,[7] Herbert Mutunzi,[2] Nico Kalisvaart, [5] Charles Sandy[2]

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My contribution to the paper was reading and making input during the publication process. I also led the primary writing of the National TB Programme drug resistant survey report where this manuscript was pulled from (see addendum 1 attached)

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Running title: Zimbabwe drug resistant TB survey

Key words: Drug resistant TB; previously treated TB; Zimbabwe; rifampicin resistant TB; MDR, Gene Xpert.
Word count: Abstract, **181**; main text, **2855; References= 29;** Tables/Figures**=5**

Abstract

Objective: To determine the prevalence of resistance to rifampicin alone; rifampicin and isoniazid, and second-line anti-TB drugs among sputum smear-positive tuberculosis patients in Zimbabwe.

Design: A health facility-based cross-sectional survey.

Results: In total, 1114 (87.6%) new and 158 (12.4%) retreatment TB patients were enrolled. MTB was confirmed by Xpert MTB/RIF among 1184 (93%) smear-positive sputum samples. There were 64 samples with Xpert MTB/RIF-determined rifampicin resistance. However, two were rifampicin susceptible on phenotypic drug susceptibility testing. The prevalence of RR-TB was [4.0% (95% CI, 2.9, 5.4%), n=42/1043) and 14.2% (95% CI, 8.9, 21.1%; n=20/141) among new and retreatment patients, respectively. The prevalence of MDR-TB was 2.0% (95% CI, 1.3, 3.1%) and 6.4% (95% CI, 2.4, 10.3%) among new and retreatment TB patients, respectively. Risk factors for RR-TB included prior TB treatment, self-reported HIV infection, travel outside Zimbabwe for ≥one month (univariate), and age <15 years. Having at least a secondary education was protective against RR-TB.

Conclusion: The prevalence of MDR-TB in Zimbabwe has remained stable since the 1994 subnational survey. However, the prevalence of RR-TB was double that of MDR-TB.

3.1 Introduction

In the modern era, *Mycobacterium tuberculosis* (MTB) drug resistance is among the key challenges in ending TB (1). In 2016, there were 600 000 new cases globally of multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin (RIF), resulting in an estimated quarter-of a million annual deaths (2). An estimated 92 629 MDR-TB cases (approximately 16% of the global burden) occurred on the African continent. However, 70% of these were not notified to health authorities, and only one-half of the countries have completed a formal drug resistance survey (DRS) (3).

Although neighbouring South Africa reports the second highest absolute number of notified rifampicin-resistant (RR) cases in the world, (defined as any resistance to rifampicin including rifampicin mono-resistance, (RMR), poly-resistance, MDR or XDR-TB), second only to India.(4,5) Studies from the north of Zimbabwe have indicated a possible increase in MDR-TB prevalence among retreatment cases.(6) Zimbabwe last conducted a representative sub-national DRS in 1994 and showed that the prevalence of MDR-TB was 1.9% (95% CI, 1.1, 3.2) and 8.3% (95% CI, 2.9, 21.8) among new and retreatment TB patients, respectively (7). At this time in the mid-1990s, HIV was rapidly becoming hyperendemic in Zimbabwe.(8) The standard TB treatment regimen then was a “short-course” 6-month regimens including RIF were yet to be adopted (this was done in 1994), antiretroviral drugs (ARVs) were unavailable, and it was questioned whether the societal costs of treating MDR-TB was worth control of the relatively small risk it presented (9). Since that time, there have been dramatic changes to the TB diagnostic and MDR-TB treatment landscapes; substantial increases in movement of economic and political migrants across borders in the Southern African region; and rapid and sustained scale-up of ARVs.

The best estimates of the burden of drug resistant TB in Africa require well-performed, population-based survey findings (10). We undertook a cross-sectional survey in 2015-2016 to: determine the prevalence among new and retreatment TB cases of Rifampicin-mono-

resistance, (defined as resistance to rifampicin with no other resistance to first line anti-TB drugs) MDR-TB, and resistance to second-line agents among those with MDR-TB. We also sought to assess the risk factors for rifampicin-resistant TB and to compare the MDR-TB estimate to that obtained in 1994 as a measure of the burden of drug-resistant TB.

3.2 Methods

3.2.1 Study design

A population-based cross-sectional study. Initially, patients in sampled health facilities were screened and diagnosed of TB using smear microscopy. Those who were smear-positive were asked to enrol in the survey as per WHO guidelines (11). The survey was conducted from August 2015-September 2016 on sputum-positive new and retreatment TB patients, regardless of age or HIV status, and not already on anti-TB therapy.

Since rifampicin resistance has conventionally been considered a proxy for MDR, and due to resource constraints, only Xpert MTB/RIF-determined rifampicin-resistant (RR) specimens proceeded to solid culture and first- and second-line DST. All patients whose samples had RR-TB strains on Xpert were re-interviewed to verify history of TB treatment.

3.2.2 Survey procedures

A survey questionnaire eliciting socio-demographic and clinical information (e.g. self-reported HIV status and history of TB) was administered to all consenting participants at enrolment. Two spot-sputum specimens were collected from consenting patients within two days of a smear-positive TB diagnosis. Sputum collection was done under the supervision of trained nurses. About 5mL of spot-sputum specimens were collected in two 50mL screw-capped falcon tubes, each containing 5mL of Cetyl-Pyridium-Chloride. This was done to maintain the integrity of the sample in case of delays (of up to 30 days) in sample transportation to the National Reference Laboratory. Each tube was labelled with a unique patient identification number (PIN). The specimens were triple-packaged in zip-lock bags to minimize spillage and

contamination and were stored at room temperature. A private courier transported the specimens to the National TB Reference Laboratory (NTBRL).

At the NTBRL, both specimens were vortexed for 15 seconds, pooled, and then split again. One specimen was tested using the Xpert MTB/RIF assay and the other was archived. A barcode reader was used to minimize transcription errors when inputting PIN numbers. In case of errors, assays were repeated using the remaining specimens from first specimens. Subsequent procedures were based upon Xpert MTB/RIF results: if RR-TB was not detected or MTB was not detected, no further procedures were performed. If RR-TB was detected, archived specimens were retrieved, decontaminated and the resultant sputum deposits were inoculated on LJ and pyruvate agar media according to standard operating procedures (12). The media were incubated at 37^{o}C and growth of MTB was observed weekly for up to 6 weeks. Part of the deposit was inoculated on LJ agar slants in 5mL cryo-vials for shipment to a Supranational TB Reference Laboratory (SRL) in Antwerp, Belgium for external quality assurance. At the NTBRL, phenotypic culture and drug susceptibility testing (CDST) was done on LJ on all MTB positive isolates using the proportion method (12). First-line DST was done for the drugs streptomycin, isoniazid, rifampicin and ethambutol (SIRE), and second-line DST for kanamycin, amikacin, ofloxacin, moxifloxacin and capreomycin. All the isolates were stored at –20^{o}C in cryo-vials with 10% glycerol. Hain Line Probe assay (LPA) (Hain LifeSciences, Nehren, Germany) was carried out on all cultures that failed to grow. Discordances between Xpert MTB/RIF RR-TB results and first-line phenotypic DST were resolved by conventional Sanger DNA sequencing of *rpoB* at the SRL. There was a 100% concordance in sensitivity and specificity between NTBRL and the SRL on the drugs kanamycin,

capreomycin, ofloxacin and rifampicin. Sensitivity and specificity of isoniazid were 90% and 89%, respectively.

3.2.3 Sampling

Sampling was done as per WHO guidelines (11). First, probability proportional to size sampling was used to select 63 of 146 national TB diagnostic sites that were functional in 2012, and 20 of 56 national TB diagnostic sites that became functional between 2012 and 2014. Within each selected diagnostic site, consecutive eligible patients were enrolled until the required number of new cases for that site was reached, or the end of the survey period was reached.

As per WHO recommendations, sample size was calculated based on new patients only; retreatment patients were sampled on convenience. For new patients, a sample size of 677 was based on the following assumptions: (i) a total national notification of 12,405, based on 2012 programme data; (ii) an absolute precision of 1% at 95% confidence interval (CI); (iii) a *priori* estimated prevalence of MDR-TB of 1.9%, based on the 1994 sub-national survey. After factoring in a design effect of two and accounting for possible losses of up to 20%, a minimum sample of 1,625 new smear-positive patients was estimated.

3.2.4 Survey and data management

A survey management team and a steering committee were established to ensure smooth implementation of the survey. A pilot survey was conducted in 10% of the sites. Three teams from the national office were trained and they later on provided on-site trainings to survey teams (TB nurses and laboratory staff) in different provinces starting with low-volume sites.

Each recruiting facility maintained a survey register which captured patient demographic and clinical data. Each patient had a PIN which was linked to all the survey tools (survey register, laboratory request form and NTBRL laboratory register). Xpert MTB/RIF and CDST results were reported to facilities to inform clinical management of patients. Quality of data was ensured through training of survey teams, cross-checking original forms during support visits by local teams and during data monitoring missions supported by staff from WHO and KNCV.

De-anonymised data were sent to the central level by a courier for double-data entry into the Census and Surveys Processing System (CSPro) database by Zimbabwe National Statistics Agency staff. Electronic data were stored in a password-protected computer and backed-up on CDs stored in a locked-file cabinet. Source documents were stored in locked-file cabinets.

3.2.4 Data analysis

Data were exported to SPSS version 20 (Chicago, Illinois, USA) for analysis. Categorical variables were summarized using frequencies. Continuous variables were summarized using means and medians as appropriate. Weighted analysis of prevalence of RR-TB and MDR-TB were done using exact sampling probabilities to adjust for sampling error due to combining two sampling methods and the capping of patient recruitment at 12 months. Odds ratios and their 95% CI for factors associated with RR-TB were calculated using the sstepwise logistic regression. Level of significance was set at $p<0.05$.

3.2.5 Ethics

This survey was approved by the Medical Research Council of Zimbabwe and the Research Council of Zimbabwe. All the participants provided written informed consent/assent prior to enrolment and collection of sputum specimens.

3.3 Results

A total of 5279 sputum smear-positive patients were notified during the survey period (Table 1). Of these, 1301 (24.6%) were initially enrolled and tested using Xpert MTB/RIF **(Figure 1).** Twenty-nine patients (2%) were excluded due to lack of survey forms and/or barcoding. The analysis population was 1272 patients, 1114 (87.6%) new and 158 (12.4%) retreatment (**Figure 1**). Of these, 766 (60.2%) were male, the median age was 34 years [(interquartile range (IQR), 27-42 years)], 699 (55.0%) self-reported a history of HIV infection, and 765 (60.1%) were recruited from urban clusters **(Table 2).** A total of 293 (23%) participants had a history of travel outside Zimbabwe of ≥one month's duration.

3.3.1 Bacteriologic results

Of the 1272 valid Xpert MTB/RIF assays, 1184 (93.1%) detected MTB. There were 44 (3.5%) new and 20 (1.6%) retreatment TB patients who had Xpert-determined RR-TB. Of these 64, 50 (78.1%) successfully grew on culture at the NTBRL. First and second-line phenotypic DST confirmed RR-TB in 48 (96%), while two cultures (4%) were susceptible to all the first-line drugs (SIRE) according to phenotypic CDST, Hain LPA (at the NTBRL), and *rpoB* gene sequencing at the SRL. Twenty-five cultures [(52.1%) (95% CI, 38.3, 65.5)] had MDR-TB; 20 demonstrated rifampicin mono-resistance (RMR); three had poly-resistance and two were rifampicin susceptible. Of the 25 MDR-

TB cultures, one (4.0%) demonstrated fluoroquinolone and aminoglycoside resistance in addition to MDR (XDR-TB).

The crude prevalence of RR-TB was [4.0% (95% CI, 2.9, 5.4%), n=42/1043] and [14.2% (95% CI, 8.9, 21.1%), n=20/141] among new and retreatment patients, respectively. The crude prevalence of MDR-TB was 2.0% [(95% CI, 1.3, 3.1%)] and [6.4% (95% CI, 2.4, 10.3%)] among new and retreatment TB patients, respectively. Among new patients, the weighted prevalence of RR-TB and MDR-TB were [4.6% (95% CI, 3.0, 6.2)] and [1.8% (95 % CI, 1.0, 2.5)] respectively.

3.3.2 Risk factors for RR-TB

In univariate analysis, a history of travel outside Zimbabwe for ≥one month [(odds ratio [(OR=1.74, 95% CI, 1.02, 2.97)] had increased odds of RR-TB. In multivariate analysis, HIV-positivity [adjusted odds ratio (aOR)=2.12 (95% CI, 1.09, 4.05)], age<15 years [aOR=6.37 (95% CI, 1.51, 26.87)], a previous history of TB treatment [aOR=3.53 (95% CI, 1.86, 6.25)] were associated with RR-TB, while having at least a secondary education was protective [(aOR=0.52; 95% CI, 0.29, 0.97)] (**Table 3).** After stratifying by type of TB patient, a positive HIV status [aOR=2.19; 95% CI, 1.07, 4.46)] and history of travel outside Zimbabwe [aOR=2.05; 95% CI, 1.05, 4.03)] were significantly associated with RR-TB among new patients (**Table 4**).

3.4 Discussion

This first nationally representative TB-DRS survey for Zimbabwe was conducted following significant socio-political and epidemiological changes in the country. We demonstrated that the prevalence of MDR-TB (defined as resistance to isoniazid and rifampicin) had remained stable since 1994 though the prevalence of RR-TB, defined as, “any resistance to rifampicin including rifampicin mono-resistance, (RMR), poly-

resistance, MDR or XDR-TB) had doubled. We also observed that the factors associated with RR-TB were a previous history of TB, HIV positivity, age <15 years, lower than secondary education and stay outside Zimbabwe for ≥a month.

The observed prevalence of MDR-TB was consistent with prevalence reported from South Africa [(2.1% (95% CI, 1.5, 2.7) and 4.6% (CI, 95%: 3.2, 6.0) among new and previously treated patients, respectively in the 2012-2014 survey] and Botswana [(2.5%, 95% CI, 1.6, 3.7) and 6.6%, 95% CI, 3.3, 11.7) among new and previously treated patients during 2007-2008)] (13,14), but was lower than prevalence recorded in both Lesotho [(3.1% (95% CI, 2.1, 4.3) and [(12.8% (95% CI, 8.8, 18.2) among previously treated patients and Namibia [(3.8% (95% CI, 2.8, 5.1) and [(16.4% (95% CI, 12.9, 20.6) among previously treated patients for the survey carried out in 2008-2009 (15,16). The increased prevalence of RR-TB could have been due to acquired resistance to rifampicin in high HIV prevalence settings like Zimbabwe. Co-infected HIV and TB individuals tend to have poor TB treatment adherence resulting in acquired drug resistance (17). Studies have shown associations between RR and HIV positive status, previous histories of TB, use of antifungals and use of rifabutin (18–20).

The findings that the previous histories of TB and stay outside Zimbabwe for ≥month (bivariate analysis) were associated with RR-TB were not surprising. The latter may stem from the fact that most Zimbabweans go to neighbouring, high TB-burden countries as economic emigrants and living conditions there may foster acquisition of RR-TB.

We do not know the reasons why attainment of secondary education was protective against RR-TB. Perhaps, attainment of secondary education is associated with better socio-economic status and positive health behavioral traits. By contrast, a study done in China showed that attainment of high school was a risk factor for RR-TB (21). Drug

resistant surveys from southern Africa did not assess the relationship between education and risk of RR-TB. Studies from high-income countries failed to show the relationship between education and risk of RR-TB. Thus, more research is needed to investigate this relationship.

This study enrolled more patients from urban than in rural areas, a consistent finding with program data in which high notifications are recorded in urban centers. This exposes geographical health inequities despite similar burden of MTB between rural and urban centers as evidenced in this study and the 2014 Zimbabwe National TB prevalence survey (22).

This survey had some limitations. First, in adopting sputum smear-positivity as our starting point, we invariably underreported RR-TB in this high HIV-burden setting given that children and HIV-positive patients often produce pauci-bacillary specimens. Second, all sputum specimens in which RR-TB was not detected did not undergo CDST. Thus, the prevalence of isoniazid mono-resistance is unknown, though it is known to be rising elsewhere in the Southern Africa Development Community (SADC) (13,23). Future DRS surveys should determine the prevalence of isoniazid-mono resistance. Third, 6% of new and 11% of retreatment sputum smear-positive specimens tested Xpert MTB/RIF-negative, raising the possibility of non-tuberculosis mycobacteria (NTM). Indeed, the prevalence of NTM is very high in Zimbabwe. During the 2014 TB prevalence survey the prevalence of NTM was estimated to be 16.9% (964/5705) of all the survey presumptive TB cases. Of the NTM isolates obtained in a convenient sample of specimens collected during the 2014 TB prevalence survey, the prevalence of clinically significant NTM such as *Mycobacteria Avium* complex (MAC) was 51.5% (41/81) (22,24). Fourth, HIV status was obtained by self-report. Incorporation of HIV testing in TB-DRS surveys could have provided crucial

information for the NTP on the relationship between HIV and drug-resistant TB (25). Lastly, we did not do multiple imputation to control for bias on the results for 14 samples which did not have DST results (no culture growths or contaminated cultures) since our data were robust. However, it would have been a useful exercise to compare potential differences in results between our models and the imputation models.

Several programmatic implications arise from this study. First, there is need to improve early and universal access to DST (in Zimbabwe and elsewhere in SADC) for at least rifampicin, in line with the WHO End TB strategy (26). Second, since isoniazid prophylactic therapy (IPT) has been scaled up in Zimbabwe with ≥20,000 PLHIV having been started on IPT by December 2015 and IPT completion rates of ≥89% have been attained (27,28), and within the context of South African isoniazid-mono resistance of [4.9%, 95% CI: 4.1%-5.8%)],(13) estimating the prevalence of and continued monitoring of isoniazid mono-resistance should be prioritized in Zimbabwe. Third, Zimbabwe has a high TB and HIV co-infection rate of about 68% (29). Continued monitoring of rifampicin and isoniazid resistance using CDST will be important for Zimbabwe given the known increased risk of prevalence of acquired drug resistance in persons with HIV/TB co-infections (30,31). Fourth, although sample size was small and should be interpreted as hypothesis-generating, we noted an increased risk of RR-TB among older children and adolescents, and warrants additional studies examining the determinants of childhood RR-TB in Zimbabwe. Lastly, we could not follow up on the treatment outcomes of this group.

In conclusion, the prevalence of MDR-TB in Zimbabwe has remained stable since the 1994 subnational survey.

Author Contributions

CS, RM, MN, JNS, JC, HM, KC, NK, BMM designed the study. JNS, JC, KC, CT, RM, MN, CS, NK analysed the data. CT, JZM and CS drafted the manuscript. BMM processed the specimens. All authors read and approved the manuscript for intellectual content. All the authors read and approved the final manuscript.

Conflict of interest statement

None declared.

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References

1. Mariandyshev A, Eliseev P. Drug-resistant tuberculosis threatens WHO's End-TB strategy. Lancet Infect Dis. 2017;17(7):674–5.

2. World Health Organisation. Global TB Report 2016. Geneva, Switzerland; 2016.

3. World Health Organisation. Global Tuberculosis Report 2017. Geneva, Switzerland; 2017.

4. World Health Organisation. World Health Organization. Global Tuberculosis Report 2015. Geneva, Switzeraland; 2015.

5. Gibson J, Donnan E, Eather G. Management of rifampicin mono-resistant tuberculosis in Queensland, Australia: a retrospective case series: RMR-TB in Queensland, Australia. Respirol Case Rep. 2018 Nov;6(8):e00366.

6. Metcalfe JZ, Makumbirofa S, Makamure B, Sandy C, Bara W, Mungofa S, et al. Drug-resistant tuberculosis in high-risk groups, Zimbabwe. Emerg Infect Dis. 2014;20(1):135–7.

7. Mwinga A. Drug - Resistant Tuberculosis in Africa. Ann N Y Acad Sci. 2006;106–12.

8. Harries AD, Hargreaves J, Kemp JR, Jindani A, Enarson D, Maher D, et al. Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. Lancet. 2001;357(9267):1519–23.

9. Schaaf H, Botha P, Beyers N, Gie R, Vermulen H, Groenwald P, et al. The 5-year outcome of multidrug resistant tuberculosis patients in the Cape Province of South Africa. Trans R Soc Trop Med Hyg. 1996;1(5):718–22.

10. Ismail NA, Mvusi L, Nanoo A, Dreyer A, Omar S V., Babatunde S, et al. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. Lancet Infect Dis. 2018;18(7):779–87.

11. World Health Organisation. Guidelines for surveillance of drug resistance in tuberculosis. 5th ed. Geneva, Switzerland; 2015.

12. Stop TB Partnership. Mycobacteriology Laboratory Manual. Geneva, Switzerland; 2014.

13. National Institute for Communicable Diseases. South African Tuberculosis Drug Resistance Survey 2012–14. Pretoria, South Africa; 2014.

14. Menzies HJ, Moalosi G, Anisimova V, Gammino V, Sentle C, Bachhuber MA, et al. Increase in anti-tuberculosis drug resistance in Botswana: results from the fourth National Drug Resistance Survey. Int J Tuberc Lung Dis. 2014;18(9):1026–33.

15. Maama-Maime LB, Mareka M, Ershova J V., Tlali TE, Kao K, Phalatse M, et al. Antituberculosis drug resistance survey in Lesotho, 2008-2009: Lessons learned. PLoS ONE. 2015;10(7):2008–9.

16. Ministry of Health and Social Services: National Tuberculosis and Leprosy Programme. Namibia National Tuberculosis Drug Resistance Survey (2008-2009). Windhoek, Namibia; 2012.

17. Daftary A, Padayatchib N, O'Donnellb, C M. Preferential adherence to antiretroviral therapy over tuberculosis (TB) treatment: a qualitative study of drug–resistant TB/HIV co–infected patients in South Africa. Glob Public Health. 2014;9(9):1107–16.

18. Meyssonnier V, Bui T Van, Veziris N, Jarlier V, Robert J. Rifampicin mono-resistant tuberculosis in France: a 2005-2010 retrospective cohort analysis. BMC Infect Dis. 2014 Jan 10;14:18.

19. Ridzon R, Whitney C, McKenna M, Taylor J, Ashkar S, Nitta A, et al. Risk Factors for Rifampin Mono-resistant Tuberculosis. Am J Respir Crit Care Med. 2013;157(6):1881–4.

20. Villegas L, Otero L, Sterling TR, Huaman MA, Van der Stuyft P, Gotuzzo E, et al. Prevalence, Risk Factors, and Treatment Outcomes of Isoniazid- and Rifampicin-Mono-Resistant Pulmonary Tuberculosis in Lima, Peru. PloS One. 2016;11(4):e0152933.

21. Yang X, Yuan Y, Pang Y, Wang B, Bai Y, Wang Y, et al. The burden of MDR/XDR tuberculosis in coastal plains population of China. PLoS ONE. 2015;10(2):1–12.

22. Ministry of Health and Child Care. Zimbabwe National Population-based Tuberculosis Prevalence survey. Harare, Zimbabwe; 2014.

23. Variava E, Martinson N. Drug-resistant tuberculosis: the rise of the monos. Lancet Infect Dis. 2018 Jul;18(7):705–6.

24. Chin'ombe N, Muzividzi B, Munemo E, Nziramasanga P. Molecular Identification of Nontuberculous Mycobacteria in Humans in Zimbabwe Using 16S Ribosequencing. Open Microbiol J. 2016;10:113–23.

25. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 5th Edition. Geneva, Switzerland; 2015.

26. STOP TB Partnership. The paradigm shift 2016-2020: Global plan to End TB. Geneva, Switzerland; 2015.

27. Takarinda KC, Harries AD, Choto RC, Mutasa-Apollo T, Chakanyuka-Musanhu C. Routine implementation of isoniazid preventive therapy in HIV-infected patients in seven pilot sites in Zimbabwe. Public Health Action. 2017;7(1):55–60.

28. Takarinda KC, Choto RC, Mutasa-Apollo T, Chakanyuka-Musanhu C, Timire C, Harries AD. Scaling up isoniazid preventive therapy in Zimbabwe: has operational research influenced policy and practice? Public Health Action. 2019;8(4):218–24.

29. World Health Organization. Global tuberculosis report 2018. 2018.

30. Mesfin YM, Hailemariam D, Biadglign S, Kibret KT. Association between HIV/AIDS and Multi-Drug Resistance Tuberculosis: A Systematic Review and Meta-Analysis. Shukla D, editor. PLoS ONE. 2014 Jan 8;9(1):e82235.

31. Rockwood N, Abdullahi LH, Wilkinson RJ, Meintjes G. Risk Factors for Acquired Rifamycin and Isoniazid Resistance: A Systematic Review and Meta-Analysis. Chatterji D, editor. PLOS ONE. 2015 Sep 25;10(9):e0139017.

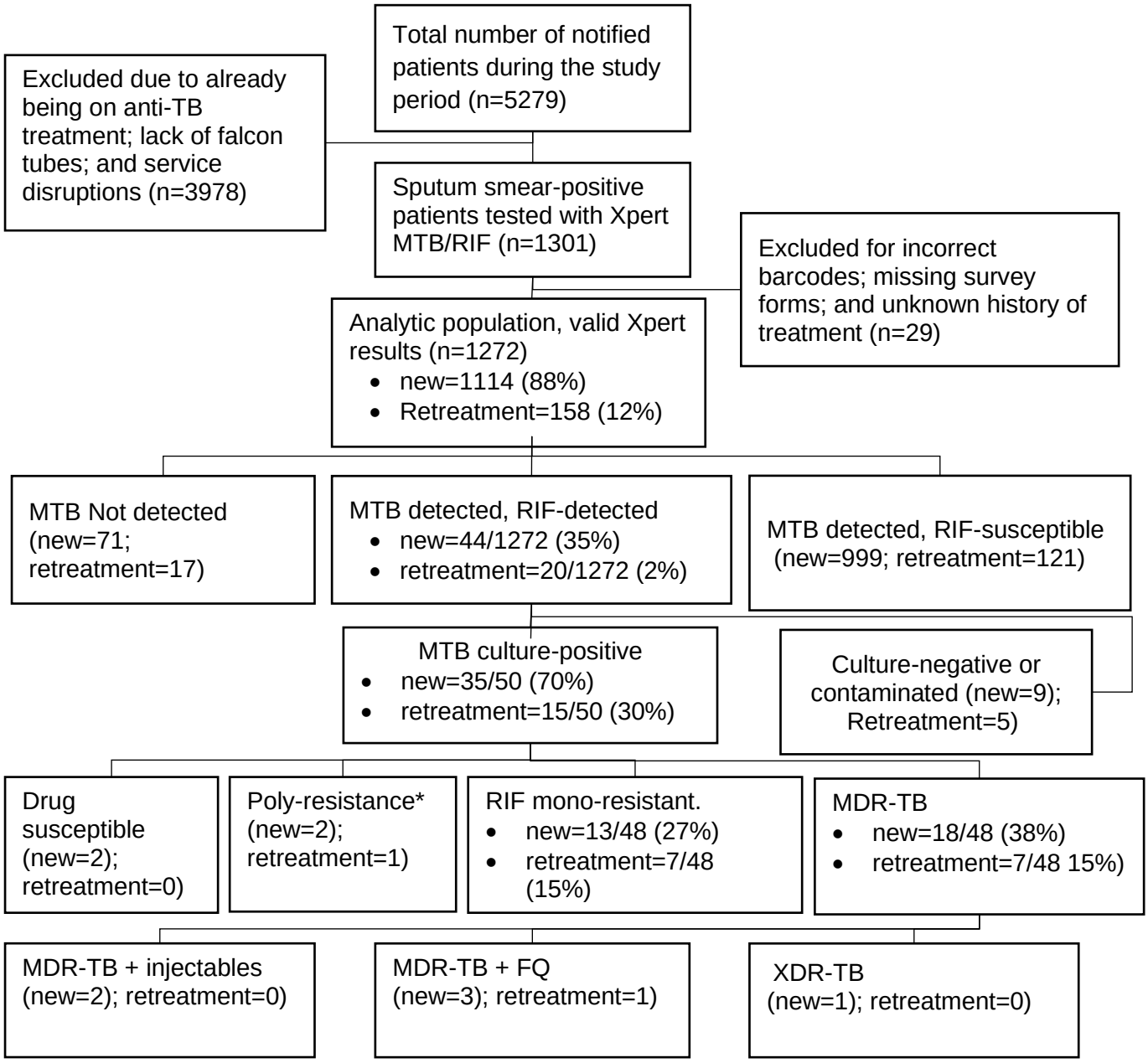

Figure 1. Flow of participants who were enrolled in the Zimbabwe DRS, 2015-2016

MDR-TB=multi-drug resistant TB; FQ=Fluoroquinolone; XDR-TB=extensively drug resistant TB; RMR=rifampicin mono-resistant TB; Poly-resistant= resistance to > one first-line anti-TB drug, other than both isoniazid and rifampicin.

Table 1. Number and proportion of participants who were enrolled in the Zimbabwe DRS, 2015-2016

Province	Total number notified during the survey period	Number of new patients	(%)	Expected number of new patients
Total	**5279**	**1114**	**(65.5)**	**1700**
Manicaland	298	135	(79.4)	170
Mashonaland Central	250	138	(73.8)	187
Mashonaland East	304	78	(65.5)	119
Mashonaland West	282	133	(65.2)	204
Matabeleland North	152	71	(54.9)	153
Matabeleland South	328	83	(46.4)	119
Midlands	300	136	(88.9)	153
Masvingo	258	93	(60.8)	153
Harare	2879	166	(46.5)	357
Bulawayo	228	81	(95.3)	85

Table 2 Socio-demographic and clinical characteristics of patients enrolled in the Zimbabwe DRS, 2015-2016

Demographic characteristics	TB patients				Total	
Total	New		Retreatment			
	N=1114	(87.6%)	N=158	(12.4%)	N=1272	(%) ‡
Sex: Male	668	(60.0)	98	(62.0)	766	(60.2)
Female	446	(40.0)	60	(38.0)	506	(39.8)
Age group (years): <15	18	(1.6)	1	(0.6)	19	(1.5)
15-24	171	(15.4)	13	(8.2)	184	(14.5)
25–34	415	(37.3)	43	(27.2)	458	(36.0)
35-44	315	(28.3)	50	(31.6)	365	(28.7)
45-54	116	(10.4)	33	(20.9)	149	(11.7)
55-64	46	(4.1)	7	(4.4)	53	(4.2)
≥65	31	(2.8)	11	(7.0)	42	(3.3)
Unknown	2	(0.2)	0	(0)	2	(0.2)
HIV status: Positive	580	(52.1)	119	(75.3)	699	(55.0)
Negative	492	(44.2)	34	(21.5)	526	(41.4)
Unknown	42	(3.7)	5	(3.2)	47	(3.6)
History of travel outside Zimbabwe						
for ≥1 month	243	(21.8)	50	(31.6)	293	(23.0)
to South Africa	166	(14.9)	32	(20.3)	198	(15.6)
Other SADC countries	62	(5.6)	17	(10.8)	79	(6.2)
To other African countries	8	(0.7)	1	(0.6)	9	(0.7)
Unknown	7	(0.6)	0	(0.0)	7	(0.6)
Marital status: Married	600	(53.9)	75	(47.5)	675	(53.1)
Never married	229	(20.6)	22	(13.9)	251	(19.7)
Divorced	177	(15.9)	33	(20.9)	210	(16.5)
Widowed	89	(8.0)	22	(13.9)	111	(8.7)
Unknown	19	(1.7)	6	(3.8)	25	(2.0)
Level of education: None	39	(3.5)	4	(2.5)	43	(3.4)
Primary	312	(28.0)	46	(29.1)	358	(28.1)
Secondary	700	(62.8)	94	(59.5)	794	(62.4)
Tertiary	55	(4.9)	13	(8.2)	68	(5.3)
Missing	8	(0.7)	1	(0.6)	9	(0.7)
Cluster location: Urban	671	(60.2)	94	(59.5)	765	(60.1)
Rural	443	(39.8)	64	(40.5)	507	(39.9)

‡=column percentages; SADC=Southern Africa Development Community; TB=tuberculosis

Table 3. Risk factors for rifampicin among smear positive patients, Zimbabwe DRS, 2015-2016

Variable	Total	RR-TB detected		OR (95% CI)		aOR 95% CI	
	1184	N=62	(5.2%)‡				
Sex: Female	466	20	(4.3)	Ref		Ref	
Male	718	42	(5.8)	1.38	(0.78, 2.52)	1.43	(0.69, 2.46)
Age group: <15	18	4	(22.2)	6.90	(1.80, 26.45) *	6.37	(1.51, 26.87) *
15-24	176	7	(4.0)	Ref		Ref	
25–34	431	19	(4.4)	1.11	(0.46, 2.70)	0.96	(0.38, 2.42)
35-44	337	22	(6.5)	1.68	(0.68, 4.77)	1.25	(0.46, 3.27)
45-54	128	5	(3.6)	0.91	(0.28, 2.92)	0.52	(0.16, 1.75)
55-64	48	3	(6.2)	1.61	(0.40, 6.47)	1.04	(0.24, 4.42)
≥65	34	2	(5.9)	0.51	(0.30, 7.60)	0.90	(0.16, 4.98)
Level of education: Primary and less	363	27	(7.4)	Ref		Ref	
Secondary and above	813	34	(4.2)	0.54	(0.31, 0.95)*	0.52	(0.29, 0.97)*
Unknown	8	1	(12.5)	1.78	(0.21, 14.99)	2.83	(0.30, 27.08)
Cluster location: Urban	714	37	(5.2)	Ref			
Rural	470	25	(5.3)	1.03	(0.58, 1.78)	0.90	(0.54, 1.71)
HIV status: Negative	508	14	(2.8)	Ref		Ref	
Positive	632	46	(7.3)	2.77	(1.46, 5.52)*	2.12	(1.09, 4.05)*
Unknown	44	2	(4.5)	1.68	(0.18, 7.70)	1.34	(0.29, 6.24)
History of any travel outside Zimbabwe							
for ≥1 month	281	22	(7.8)	1.74	(1.02, 2.97)*	1.69	(0.95, 2.99)
To South Africa	190	17	(8.9)	1.55	(0.55, 4.36)	1.49	(0.57, 4.39)
To other SADC countries	270	21	(7.8)	0.84	(0.10, 6.91)	0.87	(0.15, 6.42)
Treatment history: New	1043	42	(4.0)	Ref		Ref	
Retreatment	141	20	(14.2)	3.94	(2.11, 7.11)*	3.53	(1.86, 6.25)*

OR=odds ratio; HIV=Human immune-deficiency virus; aOR=adjusted odds ratio; SADC=Southern Africa Development Community; Ref=Reference; ‡=row percentages; *=significant

Table 4 Factors associated with rifampicin resistance among sputum smear positive patients stratified by patient type, Zimbabwe DRS, 2015-2016

	Type of TB case					
Risk factors	New (*n =1043*)		Retreatment (*n=141*)		Total (n=1184)	
	aOR	95% CI	aOR	95% CI	aOR	95% CI
Sex						
Female	1.08	0.55, 2.12	2.16	0.68, 6.82	1.43	0.69, 2.46
Male	Reference	Reference	Reference			
Age group						
<15	8.59	1.47, 50.04*	N/A	6.37	1.51, 26.87*	
15-24	Reference	Reference	Reference			
25–34	1.62	0.45, 5.84	0.28	0.05, 1.48	0.96	0.38, 2.42
35-44	2.44	0.68, 8.77	0.29	0.06, 1.40	1.25	0.46, 3.27
45-54	0.64	0.10, 4.06	0.19	0.03, 1.11	0.52	0.16, 1.75
55-64	2.43	0.45, 13.27	N/A	1.04	0.24, 4.42	
≥65	1.91	0.19, 19.80	0.20	0.02, 2.56	0.90	0.16, 4.98
Level of education						
≤Primary	Reference	Reference	Reference			
≥Secondary	0.52	0.27, 1.02	0.75	0.21, 2.67	0.52	0.29, 0.97*
Unknown	5.11	0.51, 51.25	N/A	2.83	0.30, 27.08	
HIV status						
Negative	Reference	Reference	Reference			
Positive	2.19	1.07, 4.46*	1.76	0.44, 7.09	2.12	1.09, 4.05*
Unknown	0.89	0.11, 7.25	2.72	0.21,34.71	1.34	0.29, 6.24
History of travel outside Zimbabwe						
for ≥ 1 month	2.05	1.05, 4.03*	1.09	0.37, 3.16	1.69	0.95, 2.99
Treatment history						
New						Reference
Re-treatment	3.53	1.86, 6.25*				

OR=odds ratio; HIV=Human immune-deficiency virus; aOR=adjusted odds ratio; *=significant

Chapter 4

Spatial Distribution of Mycobacterium *tuberculosis* in Metropolitan Harare, Zimbabwe

Chirenda J[1,5*]. Gwitira I[2]. Warren R[5]. Sampson SL[5]. Murwira A[2]. Masimirembwa C[1,3]. Mateveke K[1]. Duri C[4]. Chonzi P[4]. Rusakaniko S[1]. Streicher E[5].

My contribution to this paper included idea generation, design, data collation and manuscript writing. This paper has not been submitted for publication yet, but it is earmarked for publication in the PlosOne journal. The contribution to this paper was design of the study, preliminary data analysis and manuscript writing. The objective of the paper was to show that TB can occur in epidemic form especially in conditions similar to those migrants face during and after movement.

Spatial Distribution of Mycobacterium *tuberculosis* in Metropolitan Harare, Zimbabwe

Chirenda J[1,5*]. Gwitira I[2]. Warren R[5]. Sampson SL[5]. Murwira A[2]. Masimirembwa C[1,3]. Mateveke K[1]. Duri C[4]. Chonzi P[4]. Rusakaniko S[1]. Streicher E[5].

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Running Title: *Mtb* Transmission dynamics in Harare City

Key Words: Spatial distribution, TB hot spot transmission, Geospatial techniques

Abstract

Introduction

The contribution of high tuberculosis (TB) transmission pockets in propagating area-wide transmission has not been adequately described in low income and high burden settings. This study aimed to describe the presence of hotspot transmission of TB cases in Harare city from 2011 to 2012 using geospatial techniques.

Methods

Retrospective secondary data analysis cross sectional design was used. Anonymised TB patient data stored in an electronic database at Harare City Health department was retrieved, mapped and analysed using geospatial methods.

Results

A total of 12,702 TB cases were accessed and mapped on the Harare City map. Ninety (90%) of cases were new and had a high human immunodeficiency virus (HIV)/TB co-infection rate of 72% across all suburbs. Clustering of TB cases was highest in West South West, Southern and Eastern health districts. TB hot spot occurrence was restricted to the West South West health district. West South West district had an increased peri-urban population with high internal migration and inadequate social services including health facilities. These conditions were conducive for propagation of TB transmission especially in the presence of high HIV co-infection.

Conclusions and recommendations

Increased TB transmission was limited to a health district with limited health services in Harare City. To minimise spread of TB into greater Harare, there is need to improve access to TB services in the peri-urban areas.

4.1 INTRODUCTION

Tuberculosis (TB) is a respiratory infectious disease transmitted through inhalation of *Mycobacterium tuberculosis* (*Mtb*) in aerosolized droplet nuclei [1]. High population density, human immunodeficiency virus (HIV) infection, poverty, air pollution, population displacements and malnutrition increases the risk of successful TB transmission [2–4] Both host characteristics like infectiousness of the infected, number of susceptible individuals, frequency of interaction and social mixing patterns promote successful TB transmission. In addition, pathogen characteristics of virulence and mode of transmission also determine transmissibility of *Mtb* [5].

In 2017, the Africa region contributed 30% of the 30 high TB/HIV and multidrug resistant TB (MDR-TB) countries worldwide [6]. Five of these countries were from Southern Africa, a region described as the epicentre of HIV and TB infection, high migration and extreme poverty [6–8] Zimbabwe is one of the five high TB, HIV and MDR-TB burden countries from Southern Africa with a TB/HIV co-infection rate of 68% and TB incidence of 242/100,000 in 2017 [6] Despite a falling national TB incidence, the country failed to meet the Millennium Development Goal (MDG) targets of halving the TB incidence by 2017. In line with international best practice, the Zimbabwe National TB Control Program's (NTP)'s guidelines recommends ambulatory TB care for both sputum smear positive and smear negative TB patients [9].

In Zimbabwe, the two largest metropolitan cities, Harare and Bulawayo, contributed an estimated 30% of the national TB case load from 2008 to 2011 [10]. Health infrastructure in the two metropolitan cities is well established with well functional health facilities, good road and transport infrastructure and available free TB services to the patients. Causes of the persistently high TB case load have not been fully described besides the known effects of poverty and socioeconomic challenges affecting the general population since the early 2000.

The socioeconomic challenges created social instability resulting in high rural to urban migration. For Harare and Bulawayo, the increased populations was against non-expanding social services, thus increasing the risk of TB transmission from overcrowding and poverty [11]. A national program implemented in 2008 to decongest urban areas created new peri-urban settlements where there were limited health care and other social services. This internal movement of people firstly from rural areas into major urban areas in search of livelihood and later into peri-urban areas to decongest the city, may have changed the city-wide epidemiology of TB and other poverty related diseases as evidenced by the frequent outbreaks of cholera [12,13]. Pockets of high TB transmission have been known to propagate generalized TB epidemics in urban settings [14].

Methods to estimate presence of TB hotspot transmission have been limited to either use of geographical information systems (GIS) or molecular techniques or in combination of the two [15]. The utility of geospatial techniques in providing critical information on planning for TB treatment and care services has been described elsewhere but limited to high income countries [16]. Few studies have used geospatial techniques to estimate TB transmission pathways and distribution of MDR-TB clusters [15]. The aim of this study was to describe the presence of increased TB transmission zones in Harare City using geospatial techniques for the period 2011 to 2012. To our knowledge, no studies had been done to describe the spatial pattern of TB in Harare City.

4.2 MATERIALS AND METHODS

4.2.1 Setting

Harare city had an estimated total population of about two (2) million in 2012, with a good road network facilitating movement of residents between health districts. Each health district has

several suburbs. The city provides primary health care and maternity care services through its network of 12 polyclinics, 39 clinics and two infectious disease referral hospitals. Diagnosis of TB was carried out through sputum smear microscopy with Gene Xpert technology being prioritized for high risk drug resistant TB patients only. Culture and drug sensitivity testing for patients first line TB treatment failure, retreatment, rifampicin mono-resistant on Gene Xpert and other high-risk drug resistant TB patients were outsourced to the National Microbiology Reference Laboratory (NMRL) at Harare Central hospital, a tertiary government referral hospital. At the end of TB treatment, all patients within the catchment area of the two referral hospitals were discharged through the respective referral hospitals for treatment outcome determination. The South west south district was a farm converted to residential area to accommodate people who had been displaced through operation restore order. It did not have social facilities including a clinic of its own.

4.2.2 Sampling and Sample Size

Routinely collected TB diagnosis and treatment data for Harare city is stored in an Epi Info based electronic database. The Epi Info electronic database that stored all confirmed TB cases in Harare City was used to retrieve patient records for the period 2008 to 2012. Data for 2011 to 2012 was used for this analysis because data for 2008 to 2010 was incomplete, missing patient address. Data on the physical address of the patient, name of clinic providing directly observed treatment short course (DOTS), gender, age, HIV status, treatment outcome and type of TB patient, was abstracted from the database. Population projections for Harare City was obtained from the health information unit and used for estimating district specific TB prevalence by year. Patient records with incomplete physical address and other demographic characteristics were followed up to the local clinic as much as was possible.

4.2.3 Data Management

Demographic Characteristics

Microsoft excel software was used to calculate district specific TB notification for 2011-2012. District specific populations for 2011 were extrapolated from the 2002 national census using estimated growth rate and for 2012, we used the national census report.(17) Stata version 12 was used to calculate district specific prevalence, using the number of cases reported per district and the estimated district population, frequency tables of HIV status, age and sex by year and district. This provided information on whether there were any obvious differences in factors that may have affected geographical clustering of cases and TB transmission patterns.

Location data collection

Using the Epi Info electronic register, the TB patients' physical address was used to get the household location. A global positioning system (GPS) receiver was used to get the coordinates of the first ten households and the remainder of the households were mapped using Google Earth, where the streets were easily identifiable. Houses that were not on google earth were mapped using the GPS receiver through field visits. The point locations were then entered in a spreadsheet and converted into *.csv* for use in a GIS environment. The points were mapped in a GIS environment using ArcMap to visualise the TB cases.

4.2.4 Data Analyses

Estimation of spatial clustering

Prevalence of TB per health district was calculated based on the district TB case notification and estimated district population. District specific HIV prevalence was calculated from the total HIV positive divided by the total tested for HIV. Before detecting the pattern of TB prevalence in Harare over the two years, there was need to test for the presence and nature of TB spatial

autocorrelation (or dependency). To achieve this, the Global Moran's Indices was applied. The global Moran's Index was applied to confirm presence of non-random distribution of TB cases in all suburbs in Harare. The presence of spatial autocorrelation in terms of TB disease estimated the levels of TB clustering, which is indicative of hierarchical expansionary spread in urban areas and across the health districts. With an index ranging from -1 to +1, a score of zero indicated no spatial autocorrelation and a positive value indicated spatial clustering of TB cases across suburbs. A negative value showed that neighbouring suburbs were characterised by dissimilar TB cases [17]. In addition to the Global Moran's I, there was need to understand the type of spatial correlation in the distribution of TB cases by health districts. The Anselin Local Moran's I (LISA) was applied to assesses the influence of individual TB cases within surrounding health districts on other districts [18]. The results of LISA classified health districts into high values next to high-high, (HH), hotspots, (low values next to low, (LL), "cold spots" and spatial outliers (high amongst low, (HL) or vice versa, (LH). LISA was computed using the following formula [19]:

$$I_{\mathrm{i}} = \frac{\sum_{j=1}^{n} W_{ij}\,(x_i - \bar{x})\,(x_j - \bar{x})}{\frac{1}{n}\sum_{i=1}^{n}(x_{\mathrm{i}} - \bar{x})^{2}}\,, i \neq j$$

where n is the number of suburbs, x_i and x_j are the positive TB cases of suburb *i* and *j*, respectively; $\bar{x}$ is the average of the reported TB cases of all suburbs in Harare, and W_{ij} is the spatial weight matrix corresponding to the suburb pair *i* and *j*. Suburbs with high TB cases were classified as hot spots, while the ones with low TB cases were classified as cold spots. Spatial outlier represented the location where there was a mixture of high and low TB cases in neighbouring suburbs. The computation of LISA resulted in five scenarios of results: High-High, Low-Low, Low-High, High-Low, and Not Significant.

Estimation of intensity of spatial distribution

The local Getis-Ord G_i^* statistic, a hotspot analysis function in ArcGIS (Ver.10.2, ESRI Inc., CA, USA), was used to test for the intensity of incident TB cases, a measure of hotspots or cold spot occurrence TB in Harare [20,21]. The calculated ratio of the local sum of the TB cases in the vicinity of a suburb based on a five (5) metre threshold was compared to the total sum of all the TB cases in each health district to estimate intensity of incident TB cases. The intensity of incident TB cases was used to estimate transmission within the health districts. The statistical significance of a Z-score for each suburb is quantified through the presence of hot spots and cold spots of TB incidence, relative to the hypothesis of spatial uncertainty [22]. The Getis-Ord G_i^* calculates a Z-score where a significant positive Z score (Gi*) indicated hot spot phenomenon. A negative Z-score showed cold spot [20,21]. Suburbs with a Z scores > 1.96 at 99% confidence level ($p < 0.01$) were categorised as hot spots of TB. Likewise, suburbs with a Z-score of <-1.96 indicated cold spots TB clustering. We overlaid the map of health facility distribution in Harare City to that of hot spot.

4.2.5 Ethical Consideration

This study was approved by the relevant institutional review boards, Medical Research council of Zimbabwe (MRCZ), number MRCZ/A/1830 and Stellenbosch institutional review board, S16/06/106. De-identified and routinely collected secondary clinical patient data was used.

4.3 RESULTS

4.3.1 Background Characteristics of Cases Notified by Harare City, 2011-2012

Harare City notified a total of 12,702 TB patients between 2011 and 2012 (Table 1). Ninety (90%) of all TB cases were new and 4,524 (35.6%) of the patients had sputum smear positive TB. Almost 6% of the total cases died during treatment. Percentage prevalence of HIV ranged

between 65% to 77% in Central and North Western districts respectively in 2011 (Table 2). In 2012, HIV prevalence ranged between 68% to 76% in South eastern and Southern districts respectively. Prevalence of TB ranged between 158/100,000 population to 651/100,000 population in Central and Southern districts respectively in 2011. In 2012, the district with lowest TB prevalence was also the Central district and the Southern district had the highest TB prevalence, 190/100,000 population and 648/100,000 respectively. The age-groups 20-44 years old had the highest TB case load, accounting for 67% of cases in 2011 and 66% in 2012 respectively, followed by the age group >45 years old that averaged 21% for both 2011 and 2012 (Table 2). In 2012, children between 11-19 years were significantly more likely to have TB compared to 2011, 4.8% vs 5.5%, $p<0.001$. There were no differences in age-group and gender distribution by district. More males had TB (average 56.7%) than females. The proportion of sputum not done was relatively small for both 2011 and 2012, (14.4% and 8.7% respectively). Across all districts, HIV testing was high (>90%), and the HIV /TB co-infection rates averaged 72%, for both 2011 and 2012 across all districts. A combined 50% of all TB/HIV co-infected patients received antiretroviral therapy (ART), 4,280 out of the total HIV positive of 8,588. Treatment success rate was below the WHO recommended of 85%, 79.3% and 68.8% for 2011 and 2012 respectively. The number of TB patients completing treatment but not evaluated was high, 24.3% in 2011 and 15.7% in 2012 but mortality was below 10%.

Table 1 Characteristics of TB patients receiving treatment in Harare City, 2011-2012

Table 2 Tuberculosis and HIV prevalence by district, Harare City, 2011-2012

Table 3. Demographic characteristics of TB patients receiving treatment in Harare City, 2011-2012

4.3.1 Tuberculosis Prevalence in the City of Harare, 2011-2012

The prevalence of positive TB cases in Harare at the health district level for 2011 and 2012 are shown in Figure 1 a and b. Southern district had the highest TB prevalence of 651/100,000 and 648/100,000 in 2011 and 2012 respectively. Central district had the lowest TB prevalence in both 2011 and 2012, 158/100,000 and 190/100,000. The Central district has one health facility that specialises in the treatment of sexually transmitted diseases and central business district.

Fig 1a and 1b. Map showing TB prevalence rates by district, Harare City, 2011-2012

4.3.2 Spatial autocorrelation of TB Cases, Harare City, 2011-2012

In both 2011 and 2012 most of the health districts were characterised by suburbs with low (0-11) TB prevalence (Figure 2a and b). Districts located in the periphery of the greater Harare showed high TB prevalence. The global spatial autocorrelation illustrated the presence of positive spatial clustering of TB cases in Harare for both 2011 and 2012 between districts (p-value < 0.05). The clustered distribution of TB cases indicated the non-random occurrence of the cases. Suburbs in the Southern and Western areas of the city had the highest TB incidence especially the West South West (WSW), South Western and Eastern districts.

Fig 1a and b. Map showing spatial autocorrelation of TB cases between districts, Harare City, 2011-2012

4.3.3 Local spatial clustering of TB cases in Harare City

The results of Anselin's Local Moran's I analysis estimated the presence of clustering of TB cases within specific suburbs. Presence of clustering crudely measured the presence of active

transmission. The Southern and Western suburbs had TB cases that were more likely to be clustered than the rest of Harare City (Figure 2a and b). In 2011, there were more suburbs with clustering of TB cases than 2012 although at different levels. Interesting to note that despite the high incidence of TB cases in the Eastern district, the cases were more dispersed and seemed to demonstrate random occurrence.

Fig 2 a and b. Clustering of TB cases by health district, Harare City, 2011-2012

4.3.4 Intensity of TB case incidence (Hot spot)

Figure 3a and b show results of local Getis-Ord G_i^* statistic analysis. The results showed that the intensity of spatial distribution of TB cases, hotspot phenomenon, was highest in the west-south-west district of Harare city. This local pocket of TB incidence was similar for both 2011 and 2012. However, in 2012, some parts of the Eastern district showed some areas of increased intensity of TB occurrence. Within the West-South-West district, suburbs with the highest intensity of TB incidence were Budiriro, Glenview, Glen Norah and Mbare suburbs. Health facilities were concerntrated around the central districts. Within the health facilities from the South West South district, one was a specialized family planning clinic and the other were for the neighbouring districts (Fig 5).

Fig 4a and b. Hotspot occurrence of TB cases, Harare City, 2011-2012

Fig 5. Map showing the distribution of health facilities by district, Harare City, 2011-2012

4.4 DISCUSSION

Our results showed that the TB epidemiology in Harare City was mainly characterized by new patients, affecting the most economically active age group of 20-44 years, had a high TB and HIV co-infection rate of 72% and seemed to have been driven by small areas of intense TB transmission. Although the economically active population has been reported to be at highest

risk of TB and HIV infection, in Harare, proportion of the working population aged 25-49 was the highest due to the presence of employment opportunities [23]. The high proportion of new TB cases may indicate that transmission could be the most common mode of acquiring TB disease in Harare as described in studies from South Africa [5]. Previous studies from Zimbabwe also showed that majority of TB cases, more than 90% at national level were new patients [10]. In a country with adequate funding from the partners and Government and no reported shortage of anti-TB medicines, the observed continuous transmission of TB require a more focused investigations to understand the drivers of transmission. Whilst the presence of high HIV coinfection maybe driving the TB epidemic, as reported in other settings, the availability of effective interventions to reduce TB among people living with HIV in Harare City, may suggest that other factors besides HIV may have been driving the TB epidemic [10,24]

Our study showed that the Southern district had the highest TB prevalence, but the TB cases showed no spatial autocorrelation. Surveillance data from health facilities was used to calculate district specific TB prevalence. In the Southern district are three health facilities, two clinics and one infectious disease referral hospital. Also, the country's main public transport terminus is located in the Southern district. We hypothesized that the increased prevalence of unrelated TB cases could have been due to people accessing TB services in health facilities in the Southern district but staying outside the district. The contribution of this internal movement to seek health care services to the TB epidemic remains an area of research need in Zimbabwe as some studies from South Africa have reported TB transmission associated with the transport sector.(26)

Geospatial analysis of TB cases in Harare city showed that the occurrence was not random and there was differential prevalence by suburb. The suburbs with high TB prevalence and showing high intensity of TB cases, were similar in that they were both located in the periphery

of the city. In addition, they were providing services to a peri-urban population that had been created by the high rural to urban migration and efforts to decongest Harare city. The worst affected districts were the Southern, West South, West and Eastern districts. Studies describing geographic clustering of TB cases found that the most common risk factors for clustering were low socioeconomic status, homelessness, poor housing and low education attainment [27,28]. Successful transmission of TB require the presence of infectious cases that interact with susceptible uninfected individuals for a longer period of time [29] Conditions that reduce access to early TB diagnosis, early treatment and access to social services therefore promote ongoing transmission of TB. West South West and Southern districts had pockets of peri-urban populations that had no access to health care services and were relying on services from other districts, away from where they lived.

The Eastern district showed presence of clustering, an indication of relatedness of the TB cases and maybe ongoing transmission. However, there was no evidence of increased intensity of relatedness as observed in West South West district. A few reasons could explain this observation. The Eastern district was next to a rural and farming district that had adequate health facilities, with no overcrowding and relatively less poor. The rural and farming community next to the Eastern district had access to district hospital and several rural health centres servicing the communities. In addition, several small farms next to the Eastern district provided economic activity for the populations and reduced the need to travel towards the Eastern and central districts.

Assessment of TB transmission using TB notification data of children and population based surveys had used interferon gamma release assays, geospatial techniques and more recently whole genome sequencing [29] Molecular epidemiological studies have been criticized for failing to distinguish changes in transmission intensity. The use of geospatial techniques in the

African continent have been limited due to software costs and access to spatial data although they provide evidence on changes in transmission intensity [27]. Optimal methods of assessing TB transmission would be to combine molecular methods with geospatial techniques.(15) In our study, we demonstrated the utility of geospatial techniques to provide critical information for public health planning in TB programming.

Our findings from the first geospatial study of TB epidemiology in Harare brought out several issues critical for targeted TB prevention and control. Firstly, the results have demonstrated that in the absence of adequate public health services, social diseases like TB tend to occur more commonly. The increased contact or social mixing resulting from increased movement in search of social services and livelihood, may have been propagating TB transmission in Harare city and the general population [(30)]. This may explain why Harare city, has been contributing the highest TB case load in Zimbabwe. The city is one of the densely populated, contributing about 16.3% of the total population, hence facilitating increased contact between TB susceptible and infected persons [23].

Secondly, despite the decline in the national TB incidence in Zimbabwe, pockets of high TB transmission like Harare city will continue to propagate the disease at national level. Similar findings from metropolitan cities have attributed pockets of increased TB transmission, hotspots, to the continued generalized TB epidemics [14]. The mechanism of spread is believed to be increased social interaction as populations move in search of social services including health care (4,31)]. Identifying areas of increased TB transmission and plan interventions accordingly is critical for controlling the TB epidemic in Harare City. Also critical is the recognition of non-health sector developmental issues that may affect distribution TB disease.

Thirdly, our results show that use of geographical information systems (GIS) provides important information on social and environmental characteristics of TB transmission in addition to the known epidemiological characteristics [5]. With most of the available evidence of spatial epidemiology of TB having been limited to describing the TB case load in terms of prevalence over time, the use of inferential GIS statistics like the Anselin's Local Moran's I and Getis Gi statistics provided additional information on possible TB transmission dynamics (15,21,27)]. This information is important in defining areas that require targeted health and developmental interventions.

Findings from this study may have been affected by use of retrospective secondary data. This limited the time duration of follow up, as the years 2008-2010 were excluded due to missing variables on physical address. Follow up of contacts to assess the proportion of secondary cases and confirm the behavioral characteristics of TB patients reported in Harare city was not possible. An anthropological study to adequately characterize and understand the TB epidemic in Harare City is recommended [(32–34)]. It was not possible to estimate the direction of the hotspot because of the short two years duration of follow up.

Despite the above limitations, we conclude that internal migration and reduced access to public health services in Harare's peri-urban areas may have been contributing to the ongoing TB epidemic in Harare City. The authors recommend that Harare City council provide public health services to peri-urban areas to interrupt TB transmission. This could be done through establishing health posts with capacity to screen and diagnose TB using Gene Xpert as outlined by the current national health strategic plan. In addition, Harare City health should strengthen capacity of the Western, Southern and Eastern districts to provide early TB case detection and treatment to interrupt TB transmission. Working with other government sector ministries, Harare City council should ensure economic activities are available for the newly established

peri-urban settlements. This would reduce population movements in the inner city and thereby reducing potential for spreading infectious diseases like TB.

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Author Contributions

JC conceived the project, collected data and wrote the manuscript. CD and PC collected the data. IG and AM analysed the GIS component of the project. CM, KM, and SR assisted with analysis of the results. RW, SLS and EMS contributed to the writing of the article. All authors read the article.

Conflicts of interest: Authors declare no competing interests.

Data Availability

Data used in this study contain confidential physical address information and belongs to the City of Harare. This data is available from the corresponding author upon reasonable request and with explicit permission from the Harare City council.

REFERENCES

1. Wang T, Xue F, Chen Y, Ma Y, Liu Y. The spatial epidemiology of tuberculosis in Linyi City, China, 2005-2010. BMC Public Health. 2012;12:885.

2. McMichael AJ. The urban environment and health in a world of increasing globalization: issues for developing countries. Bull World Health Organ. 2000;10.

3. Kirenga BJ, Ssengooba W, Muwonge C, Nakiyingi L, Kyaligonza S, Kasozi S, et al. Tuberculosis risk factors among tuberculosis patients in Kampala, Uganda: implications for tuberculosis control. BMC Public Health. 2015;15(1):13.

4. Feenstra SG, Nahar Q, Pahan D, Oskam L, Richardus JH. A qualitative exploration of social contact patterns relevant to airborne infectious diseases in northwest Bangladesh. J Health Popul Nutr. 2013 Dec;31(4):424–34.

5. Mathema B, Andrews JR, Cohen T, Borgdorff MW, Behr M, Glynn JR, et al. Drivers of Tuberculosis Transmission. J Infect Dis. 2017 Nov 3;216(suppl_6):S644–53.

6. World Health Organization. Global tuberculosis report 2018. 2018.

7. World Health Organization. Global tuberculosis report 2017. 2017.

8. World Health Organization. Global Tuberculosis Report 2014: with CD-Rom. [Internet]. Geneva, SWITZERLAND: World Health Organization; 2014 [cited 2019 Oct 24].

9. Wright CM, Westerkamp L, Korver S, Dobler CC. Community-based directly observed therapy (DOT) versus clinic DOT for tuberculosis: a systematic review and meta-analysis of comparative effectiveness. BMC Infect Dis [Internet]. 2015 Dec [cited 2017 Oct 26];15(1).

10. Noppert G, Yang Z, Sandy C, Chirenda J. Trends of sputum-smear positive tuberculosis in Zimbabwe: 2008–2011. BMC Res Notes [Internet]. 2015 Dec [cited 2018 Feb 20];8(1).

11. Coffee MP, Garnett GP, Mlilo M, Voeten HACM, Chandiwana S, Gregson S. Patterns of Movement and Risk of HIV Infection in Rural Zimbabwe. J Infect Dis. 2005 Feb;191(s1):S159–67.

12. Luque Fernández MÁ, Mason PR, Gray H, Bauernfeind A, Fesselet JF, Maes P. Descriptive spatial analysis of the cholera epidemic 2008–2009 in Harare, Zimbabwe: a secondary data analysis. Trans R Soc Trop Med Hyg. 2011 Jan;105(1):38–45.

13. Yang C, Lu L, Warren JL, Wu J, Jiang Q, Zuo T, et al. Internal migration and transmission dynamics of tuberculosis in Shanghai, China: an epidemiological, spatial, genomic analysis. Lancet Infect Dis. 2018 Jul;18(7):788–95.

14. Dowdy DW, Golub JE, Chaisson RE, Saraceni V. Heterogeneity in tuberculosis transmission and the role of geographic hotspots in propagating epidemics. Proc Natl Acad Sci. 2012 Jun 12;109(24):9557–62.

15. Shaweno D, Karmakar M, Alene KA, Ragonnet R, Clements AC, Trauer JM, et al. Methods used in the spatial analysis of tuberculosis epidemiology: a systematic review. BMC Med. 2018 Dec;16(1):193.

16. Porter JDH. Editorial: Geographical information systems (GIS) and the tuberculosis DOTS strategy. Trop Med Int Health. 1999 Oct;4(10):631–3.

17. Zimbabwe National Statistical Agency. ZIMBABWE POPULATION CENSUS 2012. Government of Zimbabwe; 2012 p. 152.

18. Poh-Chin Lai, Fun-Mun So K-WC. Spatial Epidemiological Approaches in Disease Mapping and Analysis. 1st ed. CRC Press : New York, USA; 2009. 194 p.

19. Anselin L. Local indicators of spatial association - LISA. Geogr Anal. 1995;27(2):93–115.

20. Getis A, Ord JK. The analysis of spatial association by use of distance statistics. In: Perspectives on Spatial Data Analysis. Springer; 2010. p. 127–45.

21. Ord JK, Getis A. Local spatial autocorrelation statistics: distributional issues and an application. Geogr Anal. 1995;27(4):286–306.

22. Ord JK, Getis A. Testing for local spatial autocorrelation in the presence of global autocorrelation. J Reg Sci. 2001;41(3):411–32.

23. Mahara G, Yang K, Chen S, Wang W, Guo X. Socio-Economic Predictors and Distribution of Tuberculosis Incidence in Beijing, China: A Study Using a Combination of Spatial Statistics and GIS Technology. Med Sci. 2018;6(2):26.

24. Government of Zimbabwe. Zimbabwe Demographic and Health Survey. Zimbabwe: Zimbabwe Statistical Agency; 2015. Report No.: 2015.

25. WHO. Global TB report 2017.pdf. WHO, Head Offcie; 2017.

26. Marsha L.Feske ML, Teeter LD, Musser JM, Graviss EA. Giving TB wheels: Public transportation as a risk factor for tuberculosis transmission. Tuberculosis. 2011 Dec;Volume 91(Supplement 1):S61–S23.

27. Moonan PK, Bayona M, Quitugua TN, Oppong J, Dunbar D, Jost KC, et al. Using GIS technology to identify areas of tuberculosis transmission and incidence. Int J Health Geogr. 2004;3(1):23.

28. Peterson ML, Gandhi NR, Clennon J, Nelson KN, Morris N, Ismail N, et al. Extensively drug-resistant tuberculosis ‘hotspots’ and sociodemographic associations in Durban, South Africa. :9.

29. Yates TA, Khan PY, Knight GM, Taylor JG, McHugh TD, Lipman M, et al. The transmission of Mycobacterium tuberculosis in high burden settings. Lancet Infect Dis. 2016 Feb;16(2):227–38.

30. Churchyard G, Kim P, Shah NS, Rustomjee R, Gandhi N, Mathema B, et al. What We Know About Tuberculosis Transmission: An Overview. J Infect Dis. 2017 Nov 3;216(suppl_6):S629–35.

31. McCreesh N, Looker C, Dodd PJ, Plumb ID, Shanaube K, Muyoyeta M, et al. Comparison of indoor contact time data in Zambia and Western Cape, South Africa suggests targeting of interventions to reduce Mycobacterium tuberculosis transmission should be informed by local data. BMC Infect Dis [Internet]. 2016 Dec [cited 2017 Feb 20];16(1).

32. Erazo C, Pereira SM, Costa M da CN, Evangelista-Filho D, Braga JU, Barreto ML. Tuberculosis and living conditions in Salvador, Brazil: a spatial analysis. Rev Panam Salud Pública. 2014;36(1):24–30.

33. Alvarez-Hernández G, Lara-Valencia F, Reyes-Castro PA, Rascón-Pacheco RA. An analysis of spatial and socio-economic determinants of tuberculosis in Hermosillo, Mexico, 2000-2006. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis. 2010 Jun;14(6):708–13.

34. Izumi K, Ohkado A, Uchimura K, Murase Y, Tatsumi Y, Kayebeta A, et al. Detection of Tuberculosis Infection Hotspots Using Activity Spaces Based Spatial Approach in an Urban Tokyo, from 2003 to 2011. PloS One. 2015;10(9):e0138831.

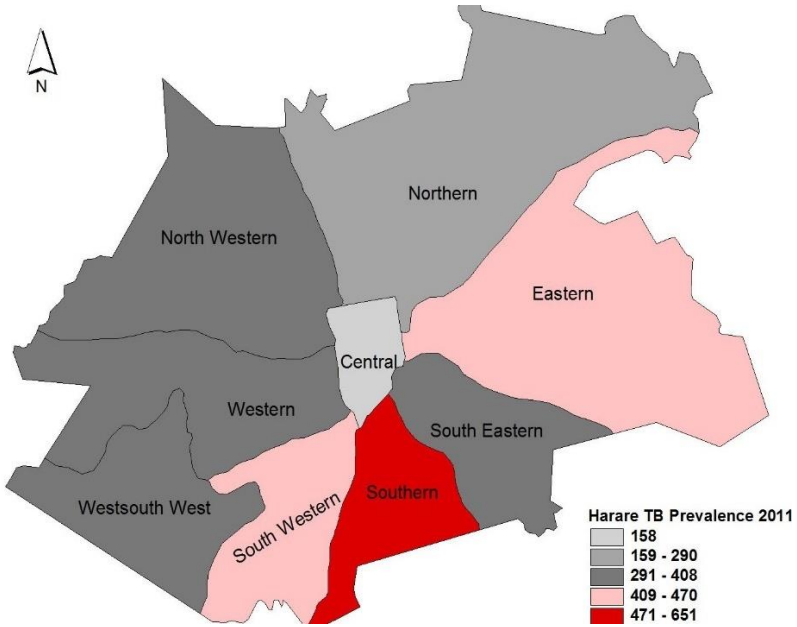


Fig 1a. 2011

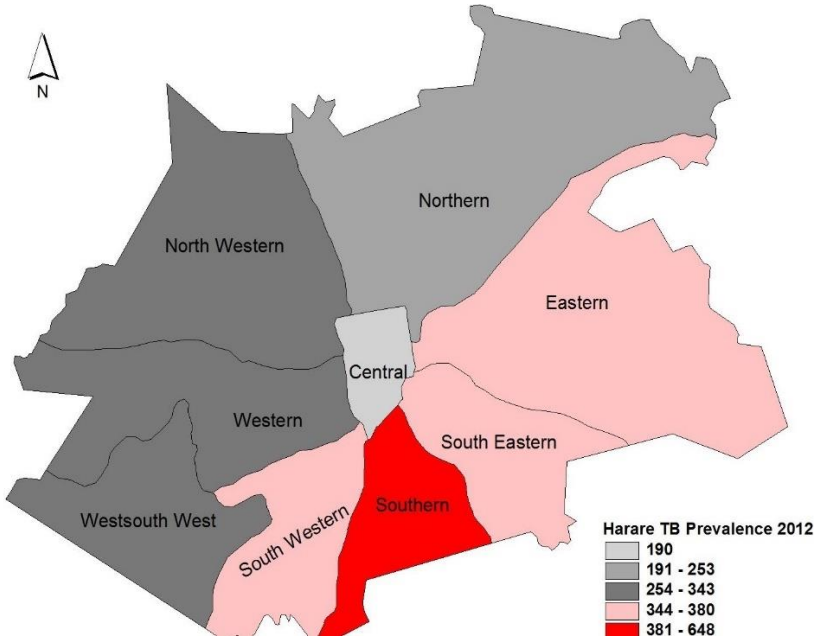
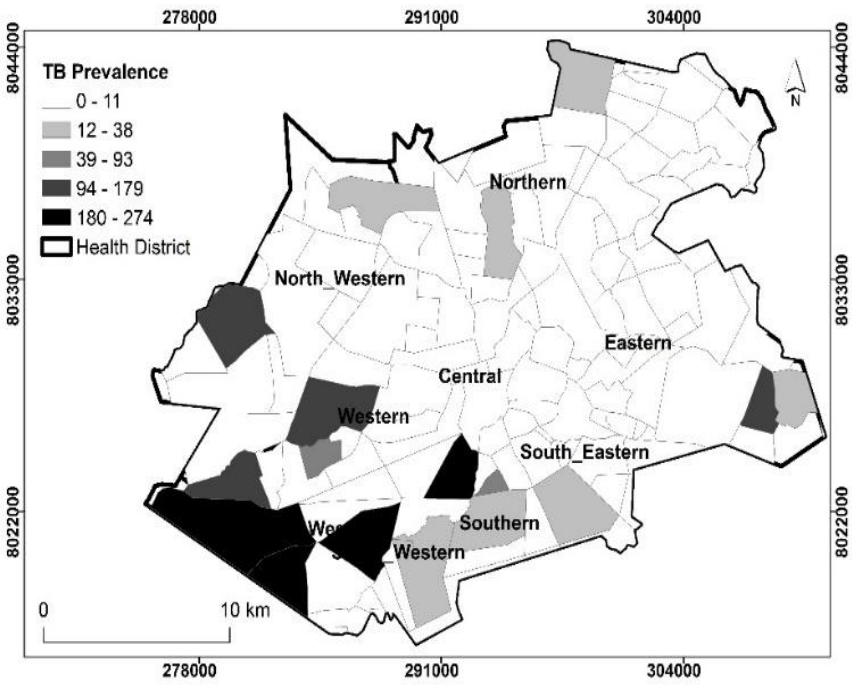


Fig 1b. 2012



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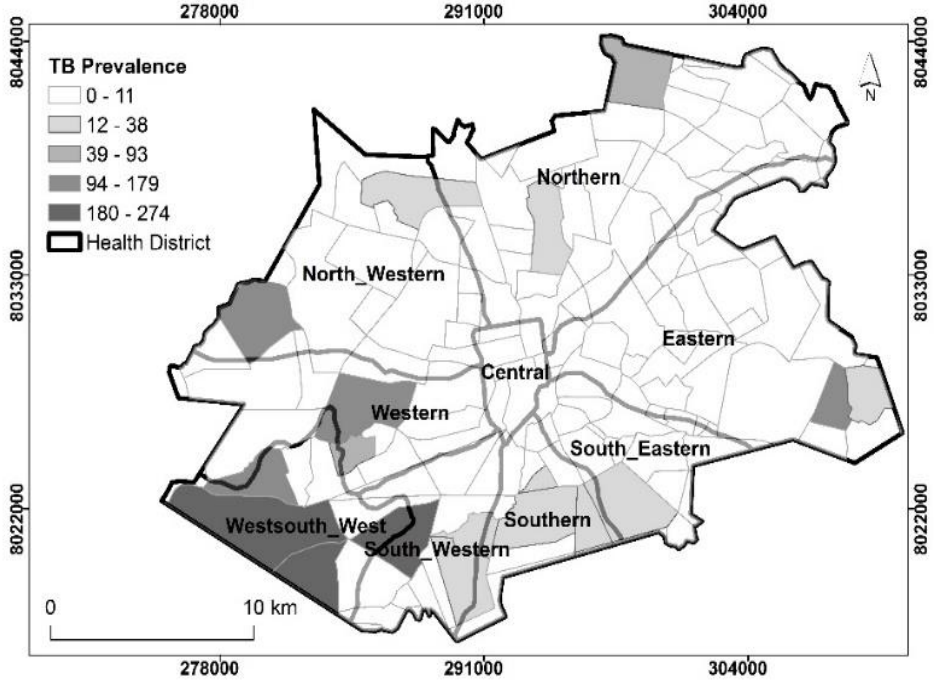

Fig 2b. 2012

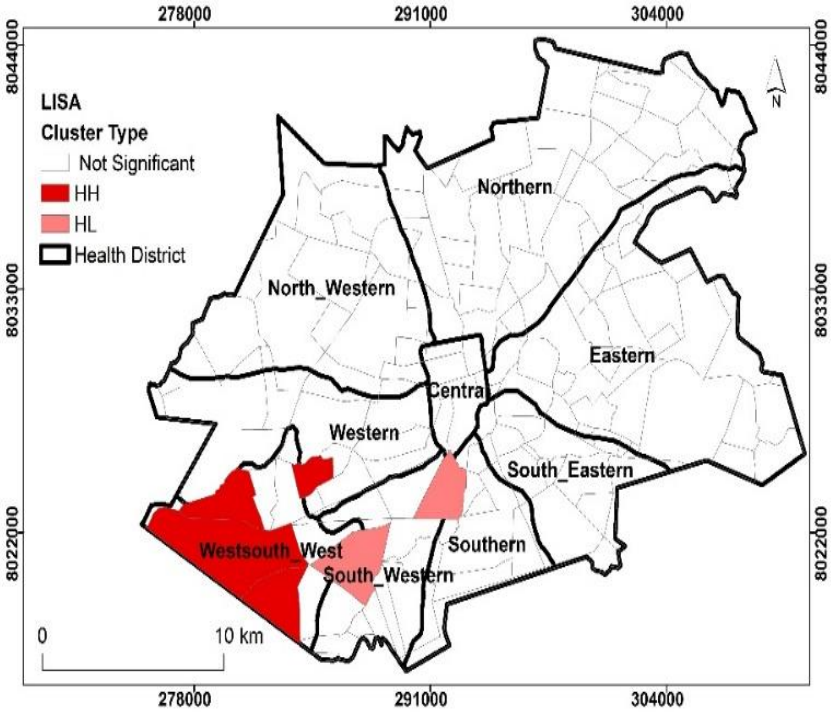

Fig 3a. 2011

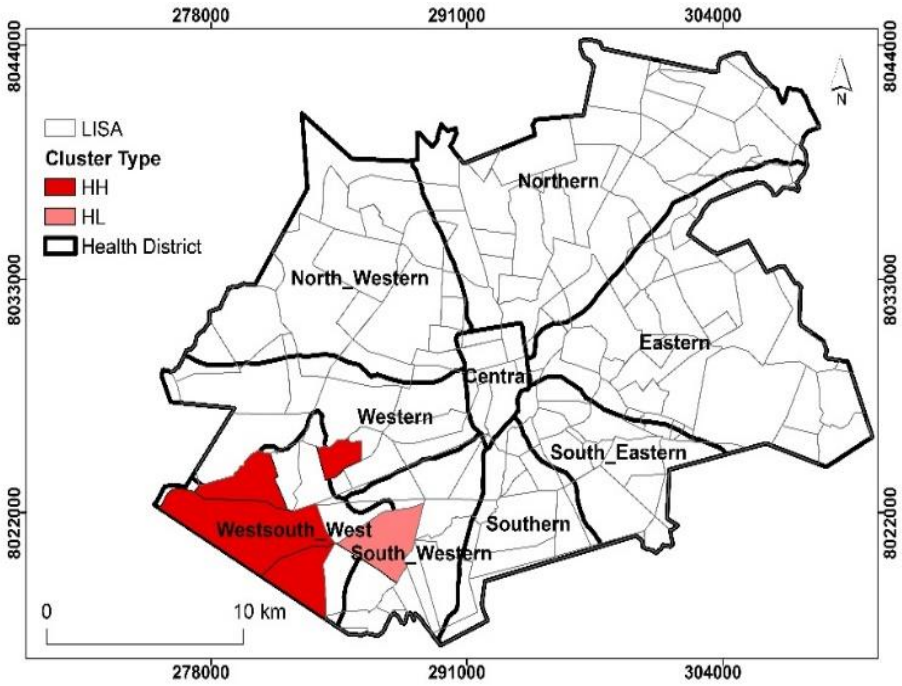

Fig 3b. 2012

Key: HH, high clustering in high prevalence areas

HL, High clustering in low prevalence areas

LL, low clustering in low prevalence areas

LH, low clustering in high prevalence areas

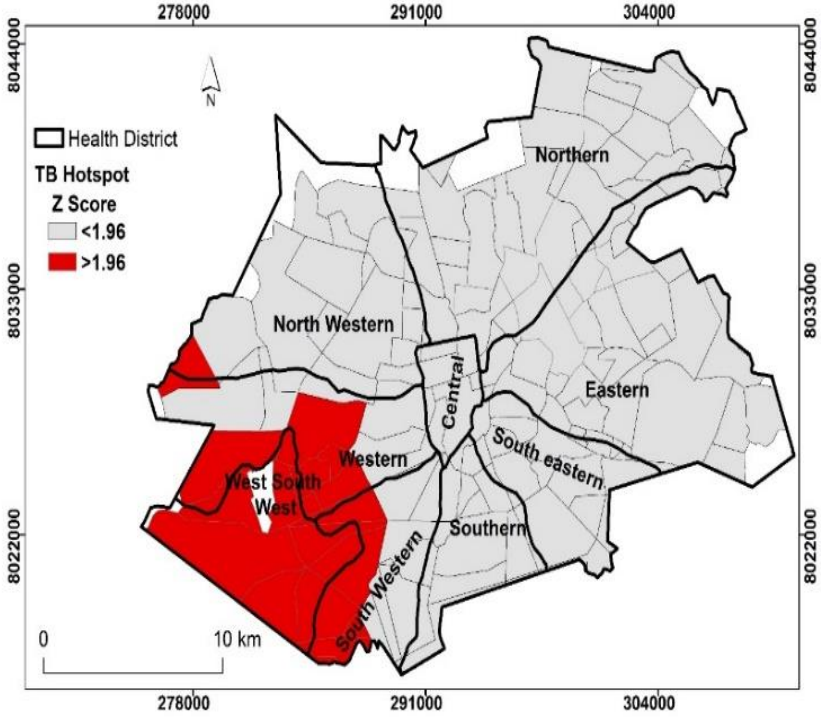

Fig 4a. 2011

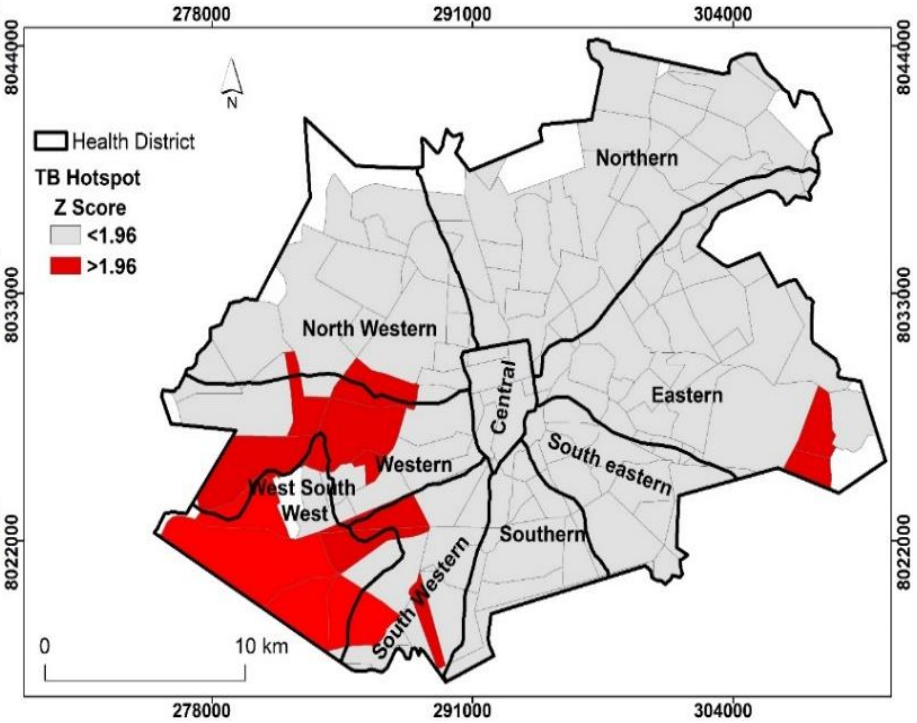

Fig 4b. 2012

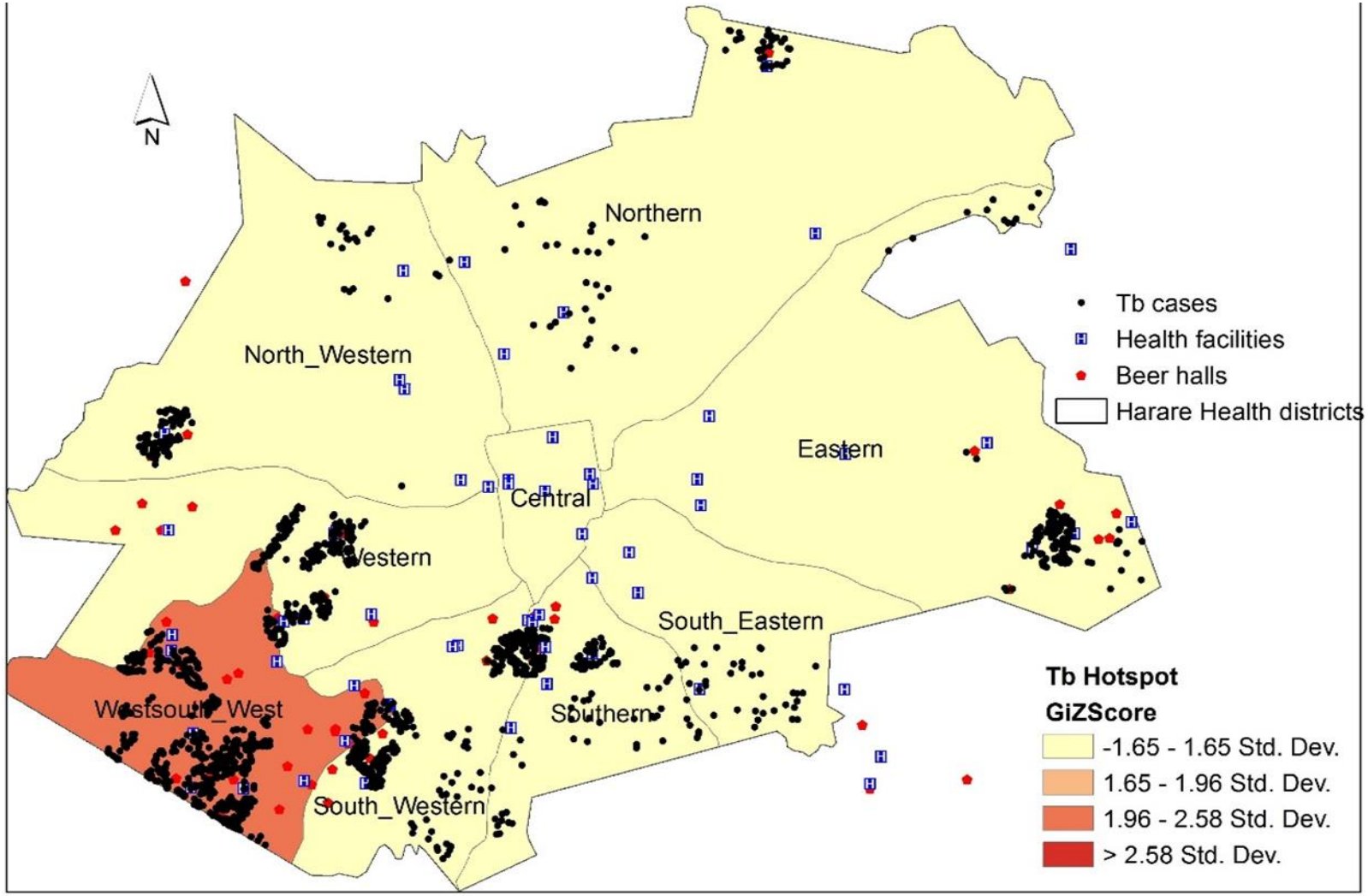

Fig 5

Table 1

Variable	**Total**	**%**	**2011**	**%**	**2012**	**%**
N	12,702	100	6,665	52.5	6,037	47.5
Patient Type: New	11,528	90.8	6,088	91.3	5,440	90.1
Retreatment	1,174	9.2	577	8.7	597	9.9
Tested for HIV: Yes	11,841	93.2	6,217	93.3	5,624	93.2
No	861	6.8	448	6.7	413	6.8
HIV positive: Yes	8,583	72.5	4,524	72.8	4,059	72.3
No	3,258	37.5	1,693	37.2	1,565	37.7
On ART: Yes	4,180	48.7	2,039	45.1	2,141	52.7
No	4,403	51.3	2,485	54.9	1,918	47.3
Treatment outcome evaluation	8,546	67.3	3,994	46.7	4,552	53.3
Treatment success	6,317	74.2	2,750	68.9	3,567	78.4
Died	506	5.9	251	6.3	255	5.6
Failed	40	0.5	25	0.6	15	0.3
Other Negative treatment outcome	1,683	19.7	968	24.2	715	15.7
Sputum smear done: Yes	8,674	68.3	4,412	64.7	4,262	72.5
No	4,028	31.7	2,412	35.3	1,616	27.5
Sputum smear result: Positive	4,524	52.2	2,362	53.5	2,162	50.7
Negative	4,150	47.8	2,050	46.5	2,100	49.3

Table 2.

Year	**2011**				**2012**			
District	Number of TB cases	Estimated Total Population	TB Prevalence/ 100,000	HIV prevalence (%)	Number of TB cases	Estimated district population	TB prevalence/100,000	% HIV prevalence
Central	91	57640	158	65	111	58281	190	73
Eastern District	686	145988	470	74	540	147612	366	70
Nothern District	300	103395	290	76	265	104545	253	73
North Western District	783	221196	354	77	737	223656	330	74
Southern District	1,073	164748	651	75	1,079	166580	648	76
South Eastern District	180	44121	408	73	161	44612	361	68
South Western	880	197012	447	76	756	199203	380	71
West	890	247597	359	73	859	250351	343	73
WSW	1,082	287197	377	70	988	290391	340	72

Table 3

Variable	2011 N (%)	2012 N (%)
Age		
<5 years	290 (4.4)	252 (4.2)
5-10 years	169 (2.5)	146 (2.4)
11-19 years	320 (4.8)	332 (5.5)
20-44 years	4468 (67.0)	4005 (66.3)
45+ years	1418 (21.3)	1302 (21.6)
Total	6665 (100)	6037 (100)
Gender		
Male	3755 (56.3)	2586 (57.2)
Female	2910 (43.7)	3451 (42.8)
Total	6665 (100)	6037 (100)

Chapter 5

Recovery of Mycobacterium tuberculosis from positive Mycobacterium growth indicator tubes stored at room temperature for up to 6 years in low income and high TB burden country

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The chapter describes the laboratory methods used to recover *Mycobacterium tuberculosis (Mtb)* in MGIT, culture and DNA extraction for molecular techniques. My contribution to this chapter was design, data collection, preliminary sub-culture into MGIT in the field, part of DNA extraction, data analysis and manuscript writing and editing

Recovery of Mycobacterium tuberculosis from positive Mycobacterium growth indicator tubes stored at room temperature for up to 6 years in low income and high TB burden country

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Abstract

Methods and Materials

Census sampling of all confirmed rifampicin / multi drug resistant (RR/MDR) TB isolates that were stored in MGIT tubes at room temperature from 2011 to 2016 were identified and retrieved. Isolates were sub-cultured on MGIT and 7H10 solid media for extraction of genomic deoxyribonucleic acid (DNA) using the Phenol/chloroform method followed by precipitation with isopropanol.

Results

A total of 248/400 (62%) isolates were successfully recovered. Recovery rates increased with declining time since last culture, with 51% for isolates stored for six years which increased to 77% for those stored for one year. Isolates that grew but were contaminated during the first sub-culture at the National Microbiology Reference Laboratory in Harare could not be recovered through decontamination because of limited resources. Decontamination was only possible during the second culture at the University of Stellenbosch.

Discussion and recommendations

Storage of *Mtb* isolates at room temperature is a viable option in low-income countries where currently recommended biobanking procedures may not be available. This low cost biobanking will facilitate research activities years later as new questions arise. Standard infection prevention and control when handling *Mtb* isolates stored under room temperature for long periods is strongly recommended as these bacteria remain viable longer than previously reported.

Key Words: *Mycobacterium tuberculosis*, Recovery rate, infection prevention and control

Running title: Recovery rate of *Mycobacterium tuberculosis* cultures stored at room temperature

5.1 Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality with a reported incidence of 10.4 million cases and 1.674 million deaths worldwide in 2016 (1). The global prevalence of Rifampicin resistant (RR) TB was 600 000, with 490 000 of these being multidrug resistant TB (MDR-TB), that is, TB resistant to at least rifampicin and isoniazid. The transmission of RR and MDR-TB remains a threat to the achievement of the global target of reducing TB deaths by 90% by 2030.(2) Effective control and management of TB is anchored on rapid, accurate and definitive diagnosis with prompt initiation of anti-TB therapy. World Health Organization recommends early diagnosis and treatment of TB will increase the likelihood of positive TB treatment outcomes, reduction of transmission risks and reduction on the risk of drug resistant *Mycobacterium tuberculosis* (*Mtb*) emergence.(3–10) There is however need to generate more evidence on this recommendation. The current recommended drug resistant TB treatment duration of 12 months or more is expensive to both patient and health care system and is associated with serious adverse drug reactions.

Development of better diagnostics and treatment modalities requires an understanding of the natural history of evolution of the bacilli, its structures and metabolism, mechanisms of drug resistance and of persistence. Such knowledge can be studied prospectively under programmatic conditions from clinical *Mtb* isolates or retrospectively using stored *Mtb* isolates.

The diagnostic algorithm for TB in Zimbabwe requires that all persons with clinical suspicion of TB will be tested using GeneXpert MTB/RIF and / or sputum smear microscopy as first line diagnostic tools. GeneXpert MTB/RIF positive sputum are submitted for culture using Mycobacterial Growth Indicator Tube (MGIT) and Lowenstein-Jensen (LJ). *Mtb* culture and drug sensitivity testing (DST) services were

available from two tertiary public health laboratories, the National Microbiology Reference Laboratory (NMRL) in Harare and the National Tuberculosis Reference Laboratory (NTRL) in Bulawayo. The NMRL provided DST services for districts in the Northern part of the country and the NTRL provided services to Southern region districts. The NTRL was the official TB reference laboratory since the time Zimbabwe introduced the national TB programme (NTP) in the 1970s whilst the NMRL started providing TB culture and drug sensitivity testing services in 2010. The laboratories process other routine clinical and research samples besides TB diagnostics. As a resource limited and high TB burden country, storage of mycobacteria culture isolates at sub-zero temperatures has been a challenge due to unavailability of -80°C freezers, space, limited constant power supply, limited skilled staff and inconsistent supply of cryovials. As a result, routine clinical mycobacteria positive cultures in MGIT tubes are kept at room temperature.

The storage of mycobacteria for long periods at -70^{0}C in enriched liquid broth without loss of metabolic activity, viability or virulence is the recommended standard protocol since its first description in 1972 (11–14). Suboptimal freezing of mycobacteria cells has been shown to result in intracellular injury leading to loss of viability despite the preservation of the cell envelope (12,15). Information on viability of mycobacteria following long term storage at room temperature is scarce. Available studies either stored sputum samples with cytylpyridirium chloride-sodium chloride or in OMNIgene preservatives for short periods of time, maximum 3 weeks (16,17). Studies on viability of *Mtb* in stored isolates at room temperature only assessed storage for 4 weeks with mixed results. In Malawi, authors compared isolates stored at room temperature and refrigeration at 4°C and at 4 weeks of follow up: only 37% vs 67% room temperature to refrigerated were recovered (18). In this study, the authors did not indicate which

medium was used to store the isolates. Other studies showed mixed results when isolates were stored for a maximum period of four weeks (19,20). No studies have described the utility of storing mycobacterium positive cultures under room temperature for future studies. This study sought to determine the recovery rate of *Mtb* from MGIT positive tubes that had been stored at room temperature from 2011, 2012 and 2015 at the NTRL and from 2015 to 2016 at the NMRL.

5.2 Methods and Materials

Positive GeneXpert RR samples were routinely cultured in BACTEC mycobacterium growth indicator tube (MGIT 960; BD) and Lowenstein-Jensen (LJ) for drug sensitivity testing. Positive MGIT tubes were then kept at room temperature.

5.2.1 Setting

A descriptive cross-sectional study was conducted on *Mtb* isolates stored at the NMRL in Harare and the NTRL in Bulawayo for the years, 2011, 2012, 2015 and 2016. Isolates from NMRL were available for the years 2015 and 2016 and isolates from the NTRL were available for the period 2011, 2012 and 2015. There were no isolates for 2016 from the NTRL because these were used for the national drug resistance survey (DRS) and therefore not available.

5.2.2. Sampling and sample size

Since the inception of programmatic management of drug resistant TB (PMDT) in 2010, the Zimbabwe National Tuberculosis and Leprosy Control Programme (NLTP) had enrolled an estimated 2,158 RR/MDR-TB cases, an under estimation according to the WHO.(21) There were two national TB reference laboratories providing drug sensitivity testing (DST), the National TB Reference Laboratory (NTRL) in the south and the National Microbiology Reference Laboratory (NMRL) in the north. All available

sample isolates were retrieved and sampled. The NTRL maintained an excel database of all samples processed and a total of 429 sample records were registered in the excel database. Of these, 130 isolates for 2009, 2013 and 2014 had been destroyed due to storage challenges. Out of the remaining 299 available isolates, a total of 259 (86.6%) were successfully retrieved and sub-culture into MGIT. The NMRL had a paper-based record of all samples ever processed. Of all the 289 registered samples, from 2015-2016, a total of 141 (48.8%) were successfully retrieved and sub-cultured in MGIT, to give a total sample size of 400 isolates.

5.2.3 Physical Storage

All isolates from the two laboratories were stored in cupboards or on the floor within the TB laboratory, at room temperature. At the NTRL, the isolates were stored in a dedicated locked room and at the NMRL isolates were in the TB culture room which had access control. There was no order in terms of year of isolate processing or by drug resistant status. Retrieving the isolates took 5 full days, 2 days at NMRL and 3 days at NTRL. At NMRL, there was no electronic database of all TB culture positive isolates and the laboratory paper register was used to identify RR/MDR-TB isolates. A health and safety officer at the NTRL ensured that face masks were used during isolate identification. There was no health and safety officer at the NMRL.

5.2.3 Laboratory Procedures

All available RR/MDR-TB isolates were sub-cultured using the BACTEC MGIT 960 system's standard procedure. Inoculation was done by transferring 0.5 ml of well mixed stored isolate into a new labelled MGIT tube, cap tightly closed, and tube mixed by inverting several times. The tubes were incubated at 37^0C until the instrument

flagged them as positive or up to a total of 42 days after which they were flagged as culture negative. Cultures were monitored daily for positivity. All positive cultures were confirmed as *Mtb* using the SD Bioline TB Ag MPT64 antigen rapid test (22).

Prior to aliquoting and shipping to the University of Stellenbosch (SU), bacterial contamination was checked for by inoculating positive MGIT culture onto blood agar plates using the isolation technique. Approximately 0.8 ml of MGIT culture was aseptically added to 0.8 ml of 25% glycerol in a cryo-vial. Prepared aliquots were kept at -20^{0}C for less than 24 hours before shipment. At SU, a volume of 0.8 ml of PANTA antibiotic mixture, (Becton Dickinson, USA), was added to the MGIT tube prior to inoculation to decontaminate the *Mtb* isolates. Cultures were kept at room temperature for a maximum of 3 days before being cultured on MGIT and 7H10 solid media under bio safety level 3 conditions utilising aseptic technique.

The purpose of the second sub-culture at Stellenbosch University was to confirm growth plus grow enough bacteria for DNA extraction. A positive 7H10 slant culture was defined as growth of at least one colony of *Mtb* confirmed by MPT64 antigen rapid test. Extraction of DNA for future molecular work was done using the Phenol/chloroform method followed by precipitation with isopropanol.(19,20) Purified DNA was re-dissolved in TE pH 8.0 buffer and stored at -20°C. Recovery and contamination rates for the first and second subculture were calculated as percentages.

5.2.4 Data management and analysis

Data was collected in Microsoft Excel which was password protected. Frequencies of number of isolates that successfully grew, number contaminated and number that

failed to grow were reported. Chi square test was used to compare proportions using Stata version 12.0

Study approval was obtained from institutional review boards for the University of Zimbabwe, College of Health Sciences (UZCHS) and SU, the Medical Research Council of Zimbabwe (MRCZ) and the Research Council of Zimbabwe (RCZ) Harare.

5.3 Results

5.3.1 Recovery Rate

A total of 400 stored MGIT cultures identified as having resistant *Mtb* strains were successfully retrieved and sub-cultured. Stored MGIT cultures from NTRL were from 2011 (n = 47), 2012 (n = 91) and 2015 (n =121) whilst those obtained from NMRL were from 2015 (n = 59) and 2016 (n =82). Of the 400 subcultures, 248 (62%) successfully grew *Mtb* and were all shipped to SU, South Africa. Overall recovery rate was lower from the NMRL, 40.4% compared to the NTRL, 62.2%, p<0.002. Recovery rate from the second sub-culture were higher and showed no difference with year of diagnosis (figure 1). Recovery rates for 2015, the only year isolates were available from both laboratories, were used to compare NMRL with NTRL. Isolates from the NTRL were more likely to grow than those from the NMRL, 77.7% vs 47.3%, p<0.001. Recovery rate for 2016 was 58.6%, but isolates were available from the NMRL only. More than 50% of sub-cultured isolates were recovered in both 2011 and 2012, (Figure 2).

5.3.2 Contamination

Contamination rates during the initial sub-culture in Harare were high, 64 (16%) and there was no capacity to decontaminate. These were excluded from isolates shipped to the SU. The contamination rates for isolates retrieved from NTRL was significantly lower than isolates retrieved from NMRL [9/172 (0.052) vs 16/76 (0.211); p = 0.0001].

Of the 248 MGIT re-sub-cultured at the SU, 218 (87.9%) were positive for *Mtb* (Figure 2b), Five were culture negative (2%), and 25 (10.1%) were contaminated. Among the contaminated cultures, 19 (76.0%) contained acid fast bacilli positive and out of these, 13 (68.4%) were successfully decontaminated, and 6 (24%) could not be successfully decontaminated. The remaining 6 did not contain acid fast bacilli and were not processed further. The total isolates successfully recovered including decontaminated isolates were 231 (93.1%) of 248 re-subcultures.

5.4 Discussion

5.4.1 Utility of Storing *Mtb* isolates at Room Temperature

This study demonstrates that where resources are limited, *Mtb* isolates can be stored at room temperature in MGIT tubes with good recovery after 6 years. Despite the current recommendation of *Mtb* storage at -70^{0}C, based on 1972 studies, (12,13,23) our findings demonstrated that *Mtb* isolates can remain viable at room temperature in MGIT tubes for a period longer than currently recommended. Without any additional conditions, Mtb isolates in low income countries can be stored at room temperature for purposes of future studies.

5.4.2 Recovery Rate

Our findings showed that more than 50% recovery was possible after 6 years of storage at room temperature. Other studies showed recovery rates of above 80% using different storage methods raging from filter paper to sub-zero freezers (Shinu *et al*. 2016) (24). However, the storage periods were for periods at most 8 weeks compared to 6 years demonstrated by in our study. Studies recommending sub-zero temperatures have argued that high temperatures and storage media used affect viability of *Mtb* strains (14). In our study, isolates were stored in the original MGIT

tubes used for culture confirmation which contains Middlebrook 7H9 broth supplemented with OADC which may indicate that the MGIT media allows *Mtb* isolates to preserve viability over long periods. The 38% of the primary isolates that failed to grow from the initial sub-cultured may be attributed to loss of viability due to storage at room temperature or contamination. Although storage at room temperature may not be optimal, this approach is more feasible in settings where sub-zero storage capacity is limited.

Whilst there may be needed to validate our findings under experimental conditions, these findings have the important implications for future biobanking of *Mtb* in low income and high burden countries. The findings provide hope towards sustaining basic science research in resource limited settings by making storage of *Mtb* isolates a more practical and feasible procedure.

5.4.3 Standard Precautions for Infection Prevention and Control

Our findings confirmed that *Mtb* strains retain their viability and possibly virulence even when stored at room temperature for about 6 years. The isolates were not stored in an orderly fashion, hampering retrieval. Both drug sensitive, RR and MDR-TB confirmed isolates were mixed in the same MGIT boxes. At the NMRL there was no health and safety officer to ensure retrieval of *Mtb* isolates was done with minimal risk to infection from stored isolates. The safety officer from NTRL provided and supervised the use of face masks to the researcher during retrieval of *Mtb* isolates. The findings have important public health implications. Firstly, there was potential risk of TB infection among laboratory workers, which has been reported to be three times more than in the general population (25) Other health care workers who may be involved in handling of the stored MGIT tubes during disposal of medical waste may have an increased risk of TB infection (26–28). Health care workers, particularly

laboratory workers, are recommended to handle TB isolates stored in MGIT according to the standard TB infection prevention and control measures to avoid contracting hospital acquired TB. The national TB control programmes (NTP) must provide adequate resources for TB infection prevention and control including creating awareness among health care workers and providing biosafe conditions for storing the isolates. Some studies have shown that health care workers have low risk perception to TB transmission (25,29).

5.5 Conclusions and Recommendations

Bio-banks have become an important means of storing specimens for future use in public health especially with the emergence of new drug resistant pathogens (30). The growing epidemic of drug resistant pathogens requires bio-banking of isolates to facilitate epidemiological surveillance and research on development of new drugs and new diagnostic technologies. Future research on stored *Mtb* isolates will facilitate understanding of mutations associated with drug resistant and therefore diagnostic tools and new drugs to treat the emerging infections. With the limited resources in low income countries, room temperature storage of *Mtb* isolates remains the feasible option to facilitate future research.

The authors propose the revision of the current *Mtb* storage protocol to include storage in MGIT tubes at room temperature in settings where facilities for minus 80 degrees are not available. New storage guidelines should include specifications on clear labelling of stored isolates and in a well contained area to minimize breakages. The storage area must be locked at all times for safety of laboratory workers and safe keeping of isolates. MGIT tubes are relatively strong and should not pose any risk to breakage, especially if the room is secure and always locked up with controlled entry.

During retrieval of the isolates, numbers in laboratory registers were used and compared with numbers on the MGIT tube. We propose that the labelling on the MGIT tubes should be clear and should indicate resistance pattern of the *Mtb* isolates. This will improve the ease of identifying the isolates in future and reduce research time. In addition, all *Mtb* isolates with RR-TB, MDR-TB, poly-resistant TB and any form of drug resistant must be stored separately in a cool dry place. Use of ordinary refrigerators where air conditioning facilities are not available could improve recovery rates.

The need for infection control procedures such as daily bench surface swabbing/ decontamination, orderly packing and storage documentation will need to be emphasized to minimise the risk of infection. During the retrieval of stored isolates, face masks were made available instead of the N95 masks. It is strongly recommended that N95 masks form part of the standard procedure when handling stored TB samples. The successful decontamination at the SU laboratory demonstrates the importance of good laboratory infrastructure for improved recovery rates in stored isolates. We therefore recommended that the NTRL should build capacity to decontaminate isolates for routine diagnostic services in order to improve case detection and early treatment initiation.

5.5.1 Limitations

Samples were stored in a haphazard manner without storage documentation and proper filing in order of resistance patterns. As a result, all stored RR/MDR-TB isolates could not be retrieved, and the time taken to retrieve the identified isolates was long. This may have affected the ability to sample all drug resistant TB cases ever reported by the two laboratories.

The two reference laboratories provided isolates for different years. The NTRL provided isolates for the period 2011, 2012 and 2015 and the NMRL provided isolates for 2015 and 2016. This affected adequate comparison between the two laboratories. Despite the lack of data for some years at the different reference laboratories, the study did show a trend in *Mtb* growth recovery rate over time.

Because we did not have capacity to decontaminate, resulting in us missing potential positives from the 38% contaminated isolates, it was not possible to compare recovery rates across laboratories given the higher contamination rates at NMRL. isolates from NMRL presented with higher contamination rates than those from NTR. It is possible that differences in efficient laboratory skills as well as good laboratory practices may have impacted on the statistically significant contamination rates between the two source laboratories. Therefore, it was not possible to estimate the optimal recovery rates of *Mtb* under the storage conditions. This therefore could have introduced bias in either direction. Optimal recovery rates for MGIT have been varied, ranging from as low as 63% to high rates of more than 90% (29). We used MDR-TB isolates which are known to have low fitness cost (31,32). This may have affected the recovery rate therefore under-estimating the utility of storing Mtb isolates at room temperature for longer periods than currently recommended. The second sub-culture at Stellenbosch University may have resulted in losses of some isolates in terms of recovery. This could have under-estimated further the recovery.

Because of the unorderly storage conditions, possibilities of cross contamination of sampled isolates could have been possible. This could have been the cause of the heavy contamination of some of the isolates and may have affected the recovery rate.

References

1. World Health Organization. Global tuberculosis report 2017. 2017.

2. Li J, Chung P-H, Leung CL, Nishikiori N, Chan EY, Yeoh E-K. The strategic framework of tuberculosis control and prevention in the elderly: a scoping review towards End TB targets. Infect Dis Poverty. 2017;6(1):70.

3. Timire C, Sandy C, Kumar AMV, Ngwenya M, Murwira B, Takarinda KC, et al. Access to second-line drug susceptibility testing results among patients with Rifampicin resistant tuberculosis after introduction of the Hain Line Probe Assay in Southern provinces, Zimbabwe. Int J Infect Dis. 2019 Apr;81:236–43.

4. Gebrezgabiher G, Romha G, Ejeta E, Asebe G, Zemene E, Ameni G. Treatment Outcome of Tuberculosis Patients under Directly Observed Treatment Short Course and Factors Affecting Outcome in Southern Ethiopia: A Five-Year Retrospective Study. Mistry N, editor. PLOS ONE. 2016 Feb 26;11(2):e0150560.

5. Belkina TV, Khojiev DS, Tillyashaykhov MN, Tigay ZN, Kudenov MU, Tebbens JD, et al. Delay in the diagnosis and treatment of pulmonary tuberculosis in Uzbekistan: a cross-sectional study. BMC Infect Dis. 2014 Dec;14(1):624.

6. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. Int J Tuberc Lung Dis. 2014 Mar 1;18(3):255–66.

7. Streicher EM, Müller B, Chihota V, Mlambo C, Tait M, Pillay M, et al. Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa. Infect Genet Evol. 2012 Jun;12(4):686–94.

8. World Health organization. Prevention and control of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. 2009 Apr. (SIXTY-SECOND WORLD HEALTH ASSEMBLY). Report No.: A62/20.

9. Vasankari T, Holmström P, Ollgren J, Liippo K, Kokki M, Ruutu P. Risk factors for poor tuberculosis treatment outcome in Finland: a cohort study. BMC Public Health. 2007 Dec;7(1):291.

10. Calver AD, Murray M, Strauss OJ, Streicher EM, Hanekom M, Liversage T, et al. Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa.

11. TORTOLI E, CICHERO P, PIERSIMONI C, SIMONETTI MT, GESU G, NISTA D. Use of BACTEC MGIT 960 for Recovery of Mycobacteria from Clinical Specimens: Multicenter Study. J CLIN MICROBIOL. 1999;37:5.

12. KIM TH, KUBICA, GP. Long-Term Preservation and Storage of Mycobacteria. Appl Microbiol. 1972;Vol. 24,(No. 3):311–7.

13. KUBICA, GP, GONTIJO-FILHO PP, KIM T. Preservation of Mycobacteria at -70°C: Persistence of Key Differential Features. J Clin Microbiol. 1977 Aug;Vol. 6,(No. 2):149–53.

14. Huang T-S, Chen Y-S, Lee SS-J, Tu H-Z, Liu Y-C. Preservation of Clinical Isolates of Mycobacterium tuberculosis Complex Directly from MGIT Culture Tubes. 2005;35(4,).

15. Shu Z, Weigel KM, Soelberg SD, Lakey A, Cangelosi GA, Lee K-H, et al. Cryopreservation of Mycobacterium tuberculosis Complex Cells. J Clin Microbiol. 2012 Nov 1;50(11):3575–80.

16. Aparna S, Moorthy KVK, Gokhale S. From microscopy centre to culture laboratory: a viable ride for mycobacteria. :3.

17. Tagliani E, Alagna R, Tafaj S, Hafizi H, Cirillo DM. Evaluation of Mycobacterium tuberculosis viability in OMNIgene-SPUTUM reagent upon multi-day transport at ambient temperature. BMC Infect Dis [Internet]. 2017 Dec [cited 2018 Apr 19];17(1).

18. Banda HT, Harries AD, Boeree MJ, Nyirenda TE, Banerjee A, Salaniponi FML. Viability of stored sputum specimens for smear microscopy and culture. :3.

19. SOOLINGEN DV, SOLL DR. Occurrence and Stability of Insertion Sequences in Mycobacterium tuberculosis Complex Strains: Evaluation of an Insertion Sequence-Dependent DNA Polymorphism as a Tool in the Epidemiology of Tuberculosis. 1991;29:9.

20. Barnett (first) R, Larson G. A Phenol–Chloroform Protocol for Extracting DNA from Ancient Samples. In: Shapiro B, Hofreiter M (eds) Ancient DNA Methods in Molecular Biology (Methods and Protocols),. Humana Press; 2012.

21. World Health Organization. Global tuberculosis report 2018. 2018.

22. Hyeon-Seon B, Mi Jung J, Shin-Seok K, Sang Woo K, Seung-Cheol K, Song-Yong P. Performance of the SD Bioline Ag MPT64 rapid test for the quick confrmation of Mtb.pdf. J Vet Sci. 2015;16(1):31–5.

23. Global laboratory initiative. Mycobacteriology laboratory manual. Stop TB Partnership; 2014.

24. Shinu P, AshokKumar Singh V, Nair A, Farooq R, Ishaq S. Long-Term Storage at −80°C: Effect on Rate of Recovery of *Mycobacterium tuberculosis* From Direct Acid-Fast Bacilli Smear-Positive Sputum Samples: Long-Term Storage of Sputum Specimens. J Clin Lab Anal. 2016 Sep;30(5):567–76.

25. von Delft A, Dramowski A, Khosa C, Kotze K, Lederer P, Mosidi T, et al. Why healthcare workers are sick of TB. Int J Infect Dis. 2015 Mar;32:147–51.

26. Du J, Pang Y, Ma Y, Mi F, Liu Y, Li L. Prevalence of tuberculosis among health care workers in tuberculosis specialized hospitals in China. J Occup Health. 2017;59(3):292–5.

27. Engelbrecht M, Janse van Rensburg A, Kigozi G, van Rensburg H. Factors associated with good TB infection control practices among primary healthcare

workers in the Free State Province, South Africa. BMC Infect Dis [Internet]. 2016 Dec [cited 2018 Feb 26];16(1).

28. Godfrey C, Tauscher G, Hunsberger S, Austin M, Scott L, et al. HIV Network Coordinating (HANC) TB Diagnostic Laboratory Working Division of AIDS, National Institute of Allergy and Infectious Diseases, A survey of tuberculosis infection control practices at the NIH/NIAID/DAIDS-supported clinical trial sites in low and middle income countries. BMC Infect Dis [Internet]. 2016 Dec [cited 2018 Feb 26];16(1).

29. Weng Y-H, Bhembe PT, Chiou H-Y, Yang C-Y, Chiu Y-W. Perceived risk of tuberculosis infection among healthcare workers in Swaziland. BMC Infect Dis [Internet]. 2016 Dec [cited 2018 Feb 26];16(1).

30. Branković I, Malogajski J, Morré SA. Biobanking and translation of human genetics and genomics for infectious diseases. Appl Transl Genomics. 2014 Jun;3(2):30–5.

31. Gagneux S. Fitness cost of drug resistance inMycobacterium tuberculosis. Clin Microbiol Infect. 2009 Jan;15:66–8.

32. Salvatore PP, Becerra MC, Abel zur Wiesch P, Hinkley T, Kaur D, Sloutsky A, et al. Fitness Costs of Drug Resistance Mutations in Multidrug-Resistant *Mycobacterium tuberculosis* : A Household-Based Case-Control Study. J Infect Dis. 2016 Jan 1;213(1):149–55.

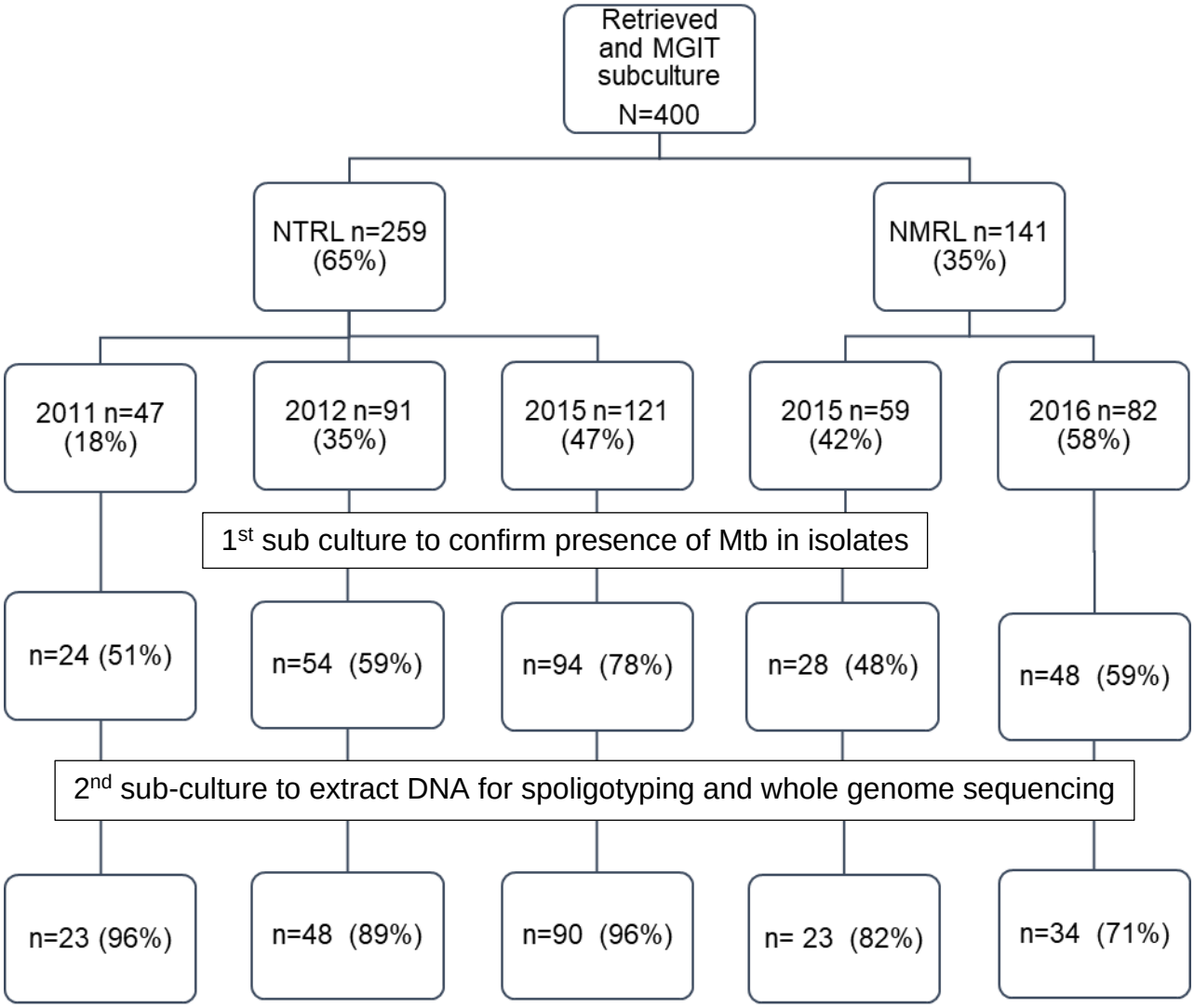

Figure 1 Showing the total number of Mtb isolates retrieved from the NTRL and NMRL laboratories, the first and second sub-culture in Harare and at the Stellenbosch University P3 laboratory respectively and final isolates used for spoligotyping and whole genome sequencing, Zimbabwe, 2011-2016

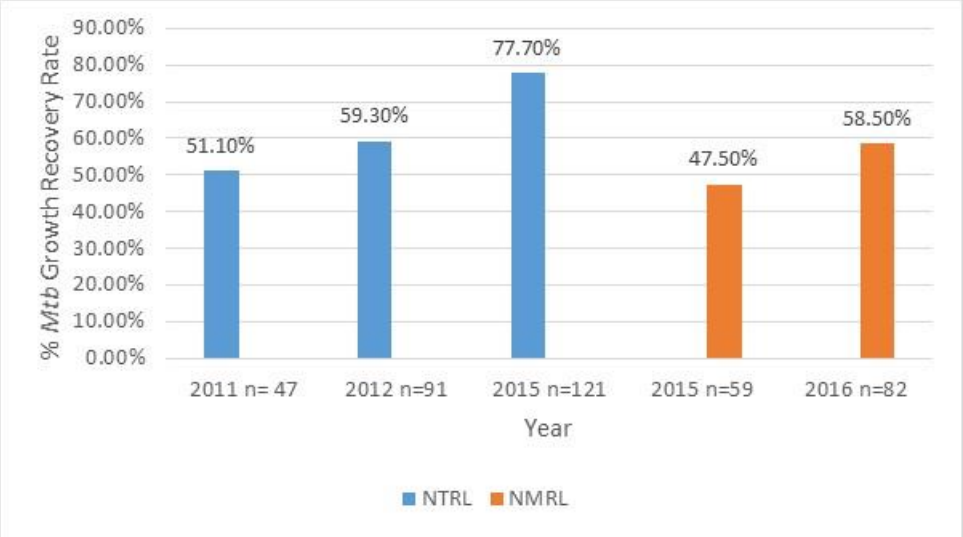

Figure 2 Percentage Mycobacterium tuberculosis *(Mtb)* growth recovery rates, by year, NMRL and NTRL, Zimbabwe 2011-2016

Chapter 6

Prevalence of Beijing Strain among rifampicin resistant /multidrug resistant tuberculosis in Zimbabwean tuberculosis patients

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This chapter is earmarked for publication in the Journal of Clinical Mycobacteriology. The paper describes the molecular epidemiology of rifampicin resistant/multidrug resistant (RR/MDR-TB) in Zimbabwe between 2011 to 2016. The results show that there is an increasing prevalence of the Beijing, T and S strains in a country where previously the LAM11_ZWE had been the predominant strain.

My contribution to this paper includes design, part of laboratory work, sample collection, DNA extraction, spoligotyping and manuscript writing

Prevalence of Beijing Strain among rifampicin resistant /multidrug resistant tuberculosis in Zimbabwean tuberculosis patients

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Abstract

Migration of Zimbabweans in the Southern Africa region could be the cause of the increasing rifampicin resistant/multidrug resistant tuberculosis (RR/MDR-TB) in Zimbabwe despite the steady decline in overall TB incidence. This study aimed to describe and compare the molecular epidemiology of RR/MDR-TB in Zimbabwe with those from the Southern African region, using spoligotyping of stored RR/MDR-TB isolates. There was a significant increase in the prevalence of Beijing, LAM11_ZWE and other T strains from 2011-2012 to 2015-20. More than forty percent (47.5%) were retreatment cases and (14.2%) died during treatment. Drug resistant (DR) TB cases from the Southern region were more likely to be retreatment, die during treatment and human immunodeficiency virus (HIV) positive compared to the Northern region, $p<0.001$. Spatial distribution of the DRTB strains showed an increased prevalence of Beijing between the Southern part of Zimbabwe, Limpopo province, Johannesburg and Botswana. The T and S strains were predominant in Botswana and the Southern region of Zimbabwe. Botswana and Angola had more Latin-American-Mediterranean (LAM), T and S strains than Beijing. We concluded that there had been an increase in the population of the Lineage 2 Beijing RR/MDR-TB strain in Zimbabwe with more from the Southern region. This increase may have been due to increased cross border movements of Zimbabweans. Cross border health systems strengthening to ensure early RR/MDR-TB diagnosis and treatment are critical to interrupt transmission in this highly mobile population with a high HIV prevalence.

6.1 Introduction

Human migration, defined as any movement of persons within or across borders for whatever reason and duration, has been responsible for the global distribution of *Mycobacterium tuberculosis (Mtb*) from as far back as more than 40,000 years ago (1,2). The development of commerce, population explosion and frequent wars had been driving human migration and with it, spread of *Mtb* (7). Global estimates in 2005 showed that 70% of the more than one billion migrants were from the low income high tuberculosis (TB) burden Southern hemisphere countries (3). In 2017, the proportion of immigrants from developing countries living in developed countries had increased to 89% (4). More important was the observation that 87% of all immigrants in developing countries were from other developing countries, an indication that regional migration was prevalent in low income countries. On the African continent, regional migration has remained relatively high in comparison to migration out of Africa and is driven by the need for livelihood (5). In the Southern Africa Development Community (SADC) region, migration patterns have been from North to South due to presence of more functional economies in South Africa and Botswana (6). Zimbabwe experienced severe socioeconomic challenges over the last two decades which resulted in increased emigration of her population to neighbouring SADC countries (7).

Despite significant research on migration and MDR-TB, there were no universally acceptable guidelines on the management of MDR-TB under migration settings. The World Health Organization (WHO) has International Regulations on MDR-TB and air travel (8). Absence of universal guidelines on the management of migration associated MDR-TB was because current evidence was based on research in low burden countries only (9). Migration and DR-TB studies from high income countries have demonstrated that immigrant populations from low income countries rarely transmit the infection to natives, had comparable treatment outcomes and

majority were due to re-activation of latent TB. (10–13). Few studies have described migration and spread of DR-TB in high burden countries and showed increased prevalence among immigrants (14,15).

Five out of the 14 SADC countries had been categorized as high tuberculosis (TB), human immunodeficiency virus (HIV) and drug resistant TB (DR-TB) by WHO (16). Surveillance data on RR/MDR-TB show that Zimbabwe notifies an average of 500 cases per year since 2010 when the country started the programmatic management of MDR-TB (PMDT). The 2015-2016 drug resistant TB survey (DRS) estimated that 4% of all new patients had RR-TB and 14.2% of retreatment patients had RR-TB (17). Compared to the 1995 DRS, the prevalence of RR-TB had doubled in 2015. The 2015 DRS report suggests that Zimbabwe continues to under-report the actual burden of RR-TB, probably due to the inadequate access to culture and drug sensitivity (CDST) services as reported elsewhere (18).

With the historically high cross border migration between Zimbabwe and other SADC countries one would expect a homogenous *Mtb* strain type circulating within the region. However, current evidence on extremely DR-TB (XDR-TB) showed that the predominant *Mtb* genotype in Zimbabwe was the LAM11_ZWE (> 20%) followed by Beijing (11,9%) (19). In South Africa and Botswana, MDR-TB strain genotypes varied by region or province but the commonest was the Beijing strain (20,21). In 2013, the Zimbabwe national TB programme observed that there were more TB deaths from the Southern region compared to the North (22). Because of the combination of high cross border migration patterns, high TB mortality and known high HIV prevalence in the Southern part of Zimbabwe, we hypothesized that the presence of foreign (not previously described) DR-TB *Mtb* strains was possibly the cause of increased mortality and probably occur in epidemic form (23,24). The aim of this study was to describe the

molecular epidemiology of DR-TB strains circulating in Zimbabwe and compare these to DR-TB strains from neighbouring SADC countries.

6.2 Materials and Methods

6.2.1. Study setting

Programmatic management of RR/MDR-TB started in 2010 in Zimbabwe. Diagnosis and treatment initiation of RR/MDR-TB was only at two specialised infectious diseases hospitals, Wilkins in the Northern region and Thorngroove in the Southern region. Between 2010 and 2015, distribution of Gene Xpert machines was limited to Harare and Bulawayo provinces plus a few mission hospitals. Two reference laboratories, the National TB Reference laboratory (NTRL) in the South and the National Microbiology Reference laboratory (NMRL), in the North were providing solid and liquid phenotypic first line drug sensitivity testing (DST) support.

Prior to 2016, the Zimbabwe NTLP used sputum smear microscopy for the diagnosis of TB. Because there were few Gene Xpert machines, only presumed high MDR-TB risk patients had sputum collected for Gene Xpert, liquid and solid phenotypic drug sensitivity testing available in Harare or Bulawayo laboratories only. High risk MDR-TB patients were all patients coming for retreatment, health care workers with confirmed TB, patients not responding to first line treatment after two to three months of treatment, people living with HIV and AIDS, confirmed TB patients from prisons and other congregate settings and contacts of MDR-TB patients. At the regional treatment centres, two sputum samples were collected, one for phenotypic DST and the other to repeat the Xpert MTB Rif assay. Patients were followed up monthly at regional DR-TB treatment centres to assess adherence, drug tolerance and presence of adverse drug reactions. Decentralization of DR-TB diagnosis, treatment initiation and follow up was from January 2016. From 2010 to 2016, the Zimbabwe National Tuberculosis and Leprosy Control

Programme (NTLP) had enrolled an estimated 2,158 RR/MDR-TB cases, which according to the WHO, was an under-estimation.(16)

A standardized short course treatment with two months rifampicin (R), isoniazid (INH), ethambutol (E), pyrazinamide (PZA) followed by four months of R and INH was used to treat susceptible TB. Sputum smear microscopy was used to monitor treatment for smear positive pulmonary TB (PTB) at two months and end of treatment. A standardized 24 month MDR-TB regimen was used for the treatment of RR/MDR-TB with monthly culture to monitor treatment response. Until February 2018 when the NTLP introduced the re-purposed drugs, the country had one 24 months individualized treatment regimen for all confirmed RR/MDR-TB patients. About 99% of all confirmed RR/MDR-TB were initiated on treatment with a low treatment success rate of less than 60% due to high mortality (16).

6.2.2 Study Population

From the 2,158 enrolled RR/MDR-TB patients from 2010 to 2016, a total of 400 isolates were identified from the two regional laboratories. These isolates were stored in MGIT tubes at room temperature, in a locked room from the NTRL and on the bench at the NMRL (Figure 1). The NTRL maintained an excel database of all samples processed. A total of 429 patient isolates were registered as confirmed RR/MDR-TB cases in the excel database from 2010 - 2016. The NTRL had destroyed 130 samples of patients recruited from 2013 to 2014 due to storage challenges. Out of the remaining 299 available isolates, 259 (86.6%) were successfully retrieved and sub-culture into MGIT. In the NMRL, a paper-based record of all samples processed was available. Out of the 289 registered isolates, 141 (48.8%) were successfully retrieved and sub-cultured in MGIT.

6.2.3 Data and Sample Collection

Using the laboratory TB registers, laboratory numbers of RR/MDR-TB isolates were used to manually identify stored samples. Data on age, HIV status and TB type was collected from the aggregated district TB/MDR-TB registers using the laboratory number. Data on treatment outcome was collected form the district DR-TB treatment registers kept regional treatment centres using the laboratory numbers.

6.2.4 Laboratory Procedures

Sub-culture of all samples was done at a University of Zimbabwe research laboratory that had a functional fume cupboard. Equal volumes of 0.8 ml each recovered isolate and glycerol were aliquoted into a 2 ml tube before shipping to the Stellenbosch University (SU) at room temperature. At SU, the samples were sub-cultured in MGIT liquid culture media and 7H10 solid media in the biosafety level (BSL) 3 laboratory.(25) A total of 157 (60.6%) and 66 (46.8%) isolates of the initially recovered isolates from NTRL and NMRL, respectively, were successfully recovered. Genomic deoxyribonucleic acid (DNA) was extracted using the phenol/chloroform method followed by precipitation with isopropanol (26). Purified DNA was re-dissolved in TE pH 8.0 buffer and stored at -20°C. Extracted DNA was assessed for concentration and quality using the Nanodrop 200C instrument. Isolates were genotyped using the internationally standardized spoligotyping method. (27)

6.2.5 Data Analysis

Spoligotype patterns, in octal and binary formats were compared to existing patterns in the international spoligotyping database SITVIT2, (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/). The spoligotype patterns were grouped into clade and family and stratified by the region and year of diagnosis. Clustering was defined as presence

of more than one strain with an identical spoligotype. Age and gender were used to describe clustering and strain diversity using Stata version 13. A literature search for RR/MDR-TB in SADC region diagnosed using spoligotyping, reporting genetic strain diversity and published in peer reviewed journals was done. Studies that used other molecular methods were excluded. A total of 6 studies were identified (21,28–32) (Table 4). Spoligotype results of DR-TB patient isolates resident in the SADC countries were abstracted and were plotted as pie charts in excel. The pie charts were imported in Arc Map on the SADC map and overlaid to the specific country.

6.2.6 Ethical Clearance

This study was approved by the Medical Research Council of Zimbabwe (MRCZ), approval number MRCZ/A/1830 and Stellenbosch University, approval number, S16/06/106.

6.3 Results

6.3.1 Demographic characteristics of RR/MDR-TB strains in Zimbabwe, 2011-2016

A total of 184/287 (64.1%), out of the initially cultured isolates had spoligotype results, 141/157 (89.8%) from the Southern region and 43/130 (33.1%) from the Northern region (Figure 6.1). There were more male (54.6%) isolates than female (Table 1). Isolates from the northern region were from older patients, mean age, 36.65 years SD ±10.87 compared to the southern region, mean age, 35.06 years, SD ±13.77. Gender and age differences between the originally sampled cohort (n=287) and successfully sub-cultured and spoligotyped cohort (n=184) were not significantly different, p=0.0965 and p=0.237 respectively. The Southern region isolates were more likely to be from retreatment patients compared to the Northern region, p<0.001. However, there was no difference in terms of HIV positivity and the likelihood of DRTB cases dying between the two regions, p=0.0788 and p=0.292 respectively (Table 2). A total of 158 RR/MDR-TB patients records had treatment outcome documented. Treatment success rate

was 65.0% and mortality was 14.2%. RR/MDR-TB patients receiving treatment from the Northern region were more likely to be cured compared to the Southern region, 28/31 (90%) vs 3/123 (2.4%), p<0.001. Cured was defined according to the WHO definitions on recording and reporting guideline document (33).

6.3.2 Characteristics of RR/MDR-TB strains circulating in Zimbabwe

Age group, gender, HIV status and treatment outcome of Latin-American-Mediterranean 11_Zimbabwe (LAM11_ZWE) strain compared to Beijing, S and T genotypes did not show any significant differences (Table 3). Mean age was similar between the Beijing and LAM11_ZWE, 35.07, SD ±10.36 vs 35.05, SD ±10.83 respectively (Table 3). The age range was similar between the S and T genotypes and the LAM11_ZWE isolates, mean age, 32.9, SD ±11.49 vs 33.32, SD ±7.53 respectively. Strain distribution did not differ with age group, p=0.542. Females were more likely to have the LAM strain, 57%, with males more likely to have the Beijing, 66.7%, LAM 4, 64.3% and T, 60% (Table 3). Among those RR/MDR-TB isolates that had a corresponding HIV test done, the Beijing, S and T1 strains were more likely to have a positive HIV result compared to LAM11_ZWE although this was not significant. Of the total 119 RR/MDR-TB patients successfully treated, only 32 (17%) were cured and 87 (47%) completed treatment.

6.3.3 Treatment outcome of RR/MDR-TB patients, Zimbabwe, 2011-2016

Isolates with Beijing and S genotypes were more likely to be from retreatment cases 25/45 (55.6%) and 11/20 (55%) respectively, compared to the LAM11_ZWE genotype that had isolates predominantly from new cases , 9/20 (45%) (Table 3). Although more strains with the Beijing genotype died, 9/45 (20%), compared to the LAM11_ZWE, 1/20 (5%), the difference in mortality was not significant, p=0.122 (Table 3).

6.3.4 Spoligotype patterns of RR/MDR-TB strains in Zimbabwe, 2011-2016

There were 21 (11.4%) clusters that ranged between 2 and 46 spoligotype patterns (Table 5). The largest cluster was the Beijing strain, 46 (25%), followed by a T, 25 (11.4%) and S, 20 (8.7%) strains. Both the Beijing and LAM11_ZWE strains predominantly occurred in the Southern region than the North, 38 (27%) vs 8 (18.6%), and 33 (16.28%) vs 12 (7.39%) respectively. Forty-one of 46 Beijing genotype isolates (89.1%) were resistant to rifampicin and 18/20 (90%) of the LAM11_ZWE and all the 20 S strains were resistant to all the 4 first line anti-TB medicines on phenotypic DST. Seventeen (9.2%) of all the isolates were rifampicin sensitive and 54 (29.67%) were sensitive to ethambutol. Of interest was the relatively high prevalence of the Euro-American S and T families in the Southern region, 21/28 (75%) and 18/20 (90%) isolates respectively (Table 6).

6.3.5 Proportion of drug resistant L2 Strain population in Zimbabwe and spatial relationship with the Southern Africa region

Table 6 show the proportion of the DR-TB L2 strain between the periods 2011-2012 and 2015-2016 for both the Northern and Southern regions. There were no differences in the proportion of DR-TB L2 strain between the northern 18/61 (30%) and southern 27/122 (22.1%) regions. No DR-TB L2 strain were diagnosed from the northern region between 2011-2012 and between 2015-2016, the northern region contributed only 8/122 (6.6%) of the cases. Proportion of the LAM11_ZWE, was low during the study period, 6/61 (9.8%) between 2011-2012 and 14/122 (11.5%) between 2015 -2016.

Figure 2 shows that the Beijing strain was more prevalent in both the Southern region and Northern regions than the LAM11_ZWE. The S and T genotypes were also common in the Southern compared to the Northern region. Comparison of the distribution of *Mtb* strains within

the SADC region using data from published literature showed that the Beijing strain was predominant in Southern region of Zimbabwe that shared borders with the South Africa's Limpopo province and Botswana. In addition, the T and S strains that were common in the South of Zimbabwe could have been due to the influence of Botswana both strains were as commonly observed as Beijing. Angola had two distinct strains, T1 and LAM 6. Most of the provinces in South Africa had more Beijing except KwaZulu-Natal that had more of LAM 4 strain (31).

6.4 Discussion

6.4.1 RR/MDR-TB strain distribution in Zimbabwe

Our study results could not categorically demonstrate spread of RR/MDR-TB L2 strain but showed that in the southern part of Zimbabwe, bordering South Africa, there was a high population of the L2 strain. South African provinces bordering Zimbabwe also had high populations of the RR/MDR-TB L2 strain. The absence of earlier (2011-2012) isolates from the Northern and 2013-2014 from the Southern regions made assessment of trends over time difficult. It would be useful to have a follow study to confirm this observation.

There were no previous studies that described molecular genetics for RR/MDR-TB in Zimbabwe at national level with the only study estimating the prevalence of XDR-TB in the Northern region among archived samples (19). In our study, the Beijing genotype was the most common genotype 45/184, (24.5%), followed by the T1, 25/184 (13.6%) and LAM11_ZWE, 20/184, (10.9%). An earlier national study that used drug susceptible TB isolates showed the predominance of a single strain, the LAM11_ZWE (34). The increased prevalence of the Beijing strain in our study compared to the previously published literature may suggest increasing circulation of the L2 strain in Zimbabwe(19). The first reason could have been due

to natural selection of the Beijing strain with improved treatment coverage as previously described (35). Secondly, given the high prevalence of the Beijing strain in countries surrounding Zimbabwe coupled with increased migration patterns of Zimbabweans within the region, this increased Beijing strain population could have been due to migration (29,31,32). The expansion of DRTB due to migration has been a subject of recent ongoing research recently (14,36,37). However, migration studies from high burden countries with increased migration patterns have been few. The temporal relationship between a period of high prevalence of LAM11_ZWE in Zimbabwe and emergence of the Beijing strain post intense migration patterns may demonstrate epidemiological linkages between Zimbabwe's TB epidemic and the South African epidemic (38,39). Our study therefore is one of the first study to describe MDR-TB transmission in areas with high MDR-TB burden and increased migration. Although majority of studies on migration and spread of TB have been limited to low incidence countries and among drug sensitive TB cases, the mechanism could apply to drug resistant TB (9).

6.4.2 Migration as a driver of Beijing transmission in Zimbabwe

Previous studies on spread of RR/MDR-TB due to migration have not been able to demonstrate successful transmission during and post migration. These studies, mainly from low burden countries receiving immigrants, have confirmed increased risk and cases of RR/MDR-TB among immigrants (13,40–43). Outbreaks of drug resistant (DR) TB have been reported, especially of the Beijing strain in populations with high HIV prevalence settings (44–46). Zimbabwe is one of the countries with a generalised HIV epidemic (47). In addition to the high HIV burden, the country has reported reduced access of laboratory services for DRTB, which may explain presence of sustained transmission of either an imported or locally available DRTB strain (48). Inadequate access to laboratory services causes delay in identifying infectious

RR/MDR-TB cases, increasing duration of exposure to susceptible individuals and therefore promote sustained transmission (49–51). The increasing Beijing genotype in Zimbabwe, with more in the Southern parts of the country may therefore be due to several reasons. First, Beijing is known to be highly virulent and its tendency to occur in epidemic form has been previously described, especially in areas with high HIV infection (24,52). Secondly, the reduced access to laboratory diagnosis for DRTB patients may have promoted the transmission of a more virulent strain like the Beijing strain over the other strains. The third reason that may support the hypothesis of migration as a major driver of RR/MDR-TB transmission in Zimbabwe is the mean age of the patients. Mean age of RR/MDR-TB patients was 35 years with a standard deviation of ±11.43 and predominantly male, which are characteristics of populations commonly migrating in search of livelihoods (6). However, because of the use of retrospective data and low successful re-culture of the samples from the Northern region, there is need for a more robust prospective study using whole genome sequencing to establish epidemiological linkages and further characterise this epidemic.

6.4.3 Characteristics of RR/MDR-TB patients in Zimbabwe

We hypothesized that the increased regular and irregular migration patterns secondary to severe socio-economic factors from mid-2000 in Zimbabwe, had promoted the spread of MDR-TB strains previously prevalent in neighbouring countries. Our study showed that RR/MDR-TB patients from the Southern region were more likely to be infected with the Beijing, T and S strains, were likely to be retreatment and with high mortality. Epidemiological HIV and AIDS studies in Zimbabwe showed that the HIV prevalence and TB mortality were higher in the Southern region compared to other parts of the country (22). The selective predominance of the Beijing strain in the Southern region could have been due to several reasons. Firstly, the populations from Zimbabwe's southern region have strong historical cultural linkages with

South Africa and Botswana and tend to freely travel across the borders (6). This may explain the presence of the T and S strains which were predominant in Botswana. The cross-border movements may have caused the importation of the S, T and Beijing strains into the region even before the onset of the economic challenges affecting Zimbabwe.

The second possible reason for the selective T, S and Beijing strains in the Southern region may have been due to the high prevalence of HIV in the region (53). The Beijing genotype has been associated with HIV co-infection and mortality (23,54). The likelihood of RR/MDR-TB cases from the Southern region being retreatment and more likely to die could have been an indication of inadequate capacity to identify and treat early drug resistant patients (48). The combination of high HIV prevalence and general national laboratory incapacity may have facilitated the sustained transmission of the Beijing strain more in the Southern part than the Northern region, especially with high volumes of migration. Inadequate access to laboratory capacity for CDST maybe promoting delay in diagnosis of RR/MDR-TB patients resulting in delayed initiation of correct treatment. This maybe facilitating ongoing transmission of untreated RR/MDR-TB.

About 9%, (4/45) of the Beijing strains showed that they had RIF sensitive TB on phenotypic DST in our study. Three possible reasons could explain this observation. Firstly, it could have been true false positives as described by Van Rie et al (55). Second, Peilei Hu et al described how phenotypic DST sometimes misses rpoB gene disputed mutations such as, Leu511Pro, Asp516Tyr, His526Asn, His526Leu, His526Cys, and Leu533Pro, picked through Xpert MTB/RIF (56–58). Third, in high TB burden settings like Zimbabwe, mixed infections could be highly likely. The World Health Organization (WHO) recommends that all RR positive results must be confirmed by culture and drug sensitivity testing to minimise inappropriate treatment of RR positive patients. Zimbabwe had limited access to culture and drug sensitivity and the

ability to confirm all false positive RIF resistance cases may be leading to inappropriate treatment of RR/MDR-TB patients.

Our study did not show any significant difference in treatment outcome between the Northern and Southern regions. This could have been due to the small sample size from the Northern region that made comparison difficult. To date, there is no evidence that show that treatment regimens differ for Beijing compared to other genotypes. However, the likelihood of the Beijing strain to progress to severe forms of drug resistance, especially in the absence of quality laboratory support is an important consideration for the Zimbabwe national TB programme to improve DRTB diagnostic capacity. Only a few, 15%, of the total RR/MDR-TB patients successfully completing treatment had a treatment outcome category of cured. This low cure rate may have been due to the previously reported low access to culture and drug sensitivity testing (CDST) of 40% in Zimbabwe (18,48).

This study has provided new knowledge on how migration in high burden settings contributes to transmission of RR/MDR-TB as previous evidence was based on migration between high and low burden countries (11). Our results agree with earlier studies on increased transmission of HIV and TB among mine and ex-mine workers including their household contacts within the SADC region (59,60). The effect of weak public health systems on the sustained transmission of imported strains will require further research especially in high MDR-TB burden settings. This will allow estimation of the contribution of migration towards transmission of MDR-TB.

6.4.4 Conclusions and key recommendations

Our study had some limitations that may have affected the strength of our findings. First, we used retrospective samples where we were not able to ensure completeness of records and collect accurate patient level data on contacts, history of migration and accurately ascertain

treatment outcome. Secondly, we had missing samples for the years 2010-2014 from the Northern region and 2013 -2014 and 2016 for the Southern region. This affected the accurate characterization of the MDR-TB epidemiology in terms of representativeness. Thirdly the inadequate laboratory capacity in Zimbabwe resulted in high contamination and poor growth from liquid culture to recover samples. This resulted in just over 50% of all recruited samples being recovered. Fourthly, the northern region laboratory capacity to diagnose RR/MDR-TB was weak and this resulted in reduced recovery of isolates. This was mainly because the laboratory only started providing RR/MDR-TB diagnostic services in 2010 and the skills base was low.

Despite these limitations, our study results confirmed that the increased presence of strains that had never been described in Zimbabwe could be due to migration. Critical recommendations to reduce incidence of MDR-TB secondary to migration could be proposed. Because of the difficulties associated with migration patterns in the SADC region, we recommended that member countries must ensure availability of diagnostic capacity for MDR-TB patients to reduce inadequate treatment from undiagnosed drug resistant strains (61). Although the cost of using molecular diagnostic techniques were prohibitive, the benefits of averted MDR-TB costs would outweigh the costs of installing molecular diagnostic methods. The improved MDR-TB patient management through early and accurate diagnosis and initiation of adequate treatment would interrupt transmission (62). We further recommend improved collaboration between countries in the SADC region in terms of implementation science research on drug treatment, host and pathogen characteristics of MDR-TB. This will harmonize the adoption of treatment regimens that are efficacious for the region since the occurrence of the MDR-TB strains is becoming homogenous.

References

1. Donoghue HD. Paleomicrobiology of Human Tuberculosis. In: Drancourt, Raoult, editors. Paleomicrobiology of Humans [Internet]. American Society of Microbiology; 2016 [cited 2019 Jul 14]. p. 113–30. A

2. World Health Organization. Tuberculosis Control in Migrant Populations Guiding Principles and Proposed Actions. 2016.

3. Carballo M, Mboup M. International migration and health. Pap Submitt Glob Comm Int Migr. 2005;

4. United Nations. Department of Economic and Social Affairs, Population Division. International Migration Report 2017. (International Migration Report 2017 (ST/ESA/SER.A/403). Report No.: International Migration Report 2017 (ST/ESA/SER.A/403.

5. Shimeles A. Migration Patterns, Trends and Policy Issues in Africa. African Development Bank Group; 2010 Dec p. 38. (Working paper series). Report No.: 119.

6. Zanamwe L, Devillard A, Zimbabwe National Statistics Agency, International Organization for Migration. Migration in Zimbabwe: a country profile 2009. Harare, Zimbabwe: Zimbabwe National Statistics Agency : International Organization for Migration; 2010.

7. Basu S, Stuckler D, Gonsalves G, Lurie M. The production of consumption: addressing the impact of mineral mining on tuberculosis in southern Africa. Glob Health. 2009;5(1):11.

8. World Health Organization, editor. Tuberculosis and air travel: guidelines for prevention and control. 3rd ed. Geneva: World Health Organization; 2008. 48 p.

9. Pareek M, Greenaway C, Noori T, Munoz J, Zenner D. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. BMC Med [Internet]. 2016 Dec [cited 2017 Jan 29];14(1).

10. Abubakar I, Aldridge RW, Devakumar D, Orcutt M, Burns R, Barreto ML, et al. The UCL–Lancet Commission on Migration and Health: the health of a world on the move. The Lancet. 2018 Dec;392(10164):2606–54.

11. Hargreaves S, Lönnroth K, Nellums LB, Olaru ID, Nathavitharana RR, Norredam M, et al. Multidrug-resistant tuberculosis and migration to Europe. Clin Microbiol Infect. 2017 Mar;23(3):141–6.

12. Bernard C, Brossier F, Sougakoff W, Veziris N, Frechet-Jachym M, Metivier N, et al. A surge of MDR and XDR tuberculosis in France among patients born in the Former Soviet Union. Eurosurveillance [Internet]. 2013 Aug 15 [cited 2019 Feb 14];18(33).

13. Cohen KA, Manson AL, Abeel T, Desjardins CA, Chapman SB, Hoffner S, et al. Extensive global movement of multidrug-resistant *M. tuberculosis* strains revealed by whole-genome analysis. Thorax. 2019 Sep;74(9):882–9.

14. Cain KP, Marano N, Kamene M, Sitienei J, Mukherjee S, Galev A, et al. The movement of multidrug-resistant tuberculosis across borders in East Africa needs a regional and global solution. PLoS Med. 2015;12(2):e1001791.

15. Matteelli A, Lönnroth K, Mosca D, Getahun H, Centis R, D'Ambrosio L, et al. Cameroon's multidrug-resistant tuberculosis treatment programme jeopardised by cross-border migration. Eur Respir J. 2016 Feb;47(2):686–8.

16. World Health Organization. Global tuberculosis report 2018. 2018.

17. Timire C, Metcalfe JZ, Chirenda J, Scholten JN, Manyame-Murwira B, Ngwenya M, et al. Prevalence of drug-resistant tuberculosis in Zimbabwe: a health facility-based cross-sectional survey. Int J Infect Dis. 2019 Jul;S1201971219303066.

18. Timire C, Takarinda KC, Harries AD, Mutunzi H, Manyame-Murwira B, Kumar AMV, et al. How has the Zimbabwe mycobacterial culture and drug sensitivity testing system among re-treatment tuberculosis patients functioned during the scale-up of the Xpert MTB/RIF assay? Trans R Soc Trop Med Hyg. 2018 Jun 1;112(6):285–93.

19. Sagonda T, Mupfumi L, Manzou R, Makamure B, Tshabalala M, Gwanzura L, et al. Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resistant Tuberculosis Isolates in Zimbabwe. Tuberc Res Treat. 2014;2014:1–8.

20. Glynn JR, Crampin AC, Traore H, Yates MD, Mwaungulu FD, Ngwira BM, et al. *Mycobacterium tuberculosis* Beijing Genotype, Northern Malawi. Emerg Infect Dis. 2005 Jan;11(1):150–3.

21. Perdigão J, Clemente S, Ramos J, Masakidi P, Machado D, Silva C, et al. Genetic diversity, transmission dynamics, and drug resistance of Mycobacterium tuberculosis in Luanda, Angola. Int J Mycobacteriology. 2016 Dec;5:S38–9.

22. Takarinda KC, Sandy C, Masuka N, Hazangwe P, Choto RC, Mutasa-Apollo T, et al. Factors Associated with Mortality among Patients on TB Treatment in the Southern Region of Zimbabwe, 2013. Tuberc Res Treat. 2017;2017.

23. Caws M, Thwaites G, Stepniewska K, Lan NTN, Duyen NTH, Phuong NT, et al. Beijing Genotype of Mycobacterium tuberculosis Is Significantly Associated with Human Immunodeficiency Virus Infection and Multidrug Resistance in Cases of Tuberculous Meningitis. J Clin Microbiol. 2006 Nov 1;44(11):3934–9.

24. Drobniewski F, Balabanova Y, Nikolayevsky V, Ruddy M, Kuznetzov S, Zakharova S, et al. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. JAMA. 8;293(22):2726-31.; 2005.

25. Pardini M, Varaine F, Bonnet M, Orefici G, Oggioni MR, the LONG-DRUG Study Group, et al. Usefulness of the BACTEC MGIT 960 System for Isolation of Mycobacterium tuberculosis from Sputa Subjected to Long-Term Storage. J Clin Microbiol. 2007 Feb 1;45(2):575–6.

26. Warren RM, Sampson SL, Richardson M, Van Der Spuy GD, Lombard CJ, Victor TC, et al. Mapping of IS6110 flanking regions in clinical isolates of Mycobacterium tuberculosis demonstrates genome plasticity. Mol Microbiol. 2000;37(6):1405–1416.

27. Kamerbeek J, Schouls L, Kolk A, Van Agterveld M, Van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol. 1997;35(4):907–914.

28. Marais BJ, Mlambo CK, Rastogi N, Zozio T, Duse AG, Victor TC, et al. Epidemic Spread of Multidrug-Resistant Tuberculosis in Johannesburg, South Africa. J Clin Microbiol. 2013 Jun 1;51(6):1818–25.

29. Said HM, Kock MM, Ismail NA, Mphahlele M, Baba K, Omar SV, et al. Molecular Characterization and Second-Line Antituberculosis Drug Resistance Patterns of Multidrug-Resistant Mycobacterium tuberculosis Isolates from the Northern Region of South Africa. J Clin Microbiol. 2012 Sep 1;50(9):2857–62.

30. Maningi NE, Daum LT, Rodriguez JD, Said HM, Peters RPH, Sekyere JO, et al. Multi- and Extensively Drug Resistant *Mycobacterium tuberculosis* in South Africa: a Molecular Analysis of Historical Isolates. Kraft CS, editor. J Clin Microbiol. 2018 Mar 7;56(5):e01214-17.

31. Gandhi NR, Brust JCM, Moodley P, Weissman D, Heo M, Ning Y, et al. Minimal Diversity of Drug-Resistant *Mycobacterium tuberculosis* Strains, South Africa1. Emerg Infect Dis. 2014 Mar;20(3):394–401.

32. Mogashoa T, Melamu P, Ley SD, Streicher EM, Iketleng T, Kelentse N, et al. Genetic diversity of Mycobacterium tuberculosis strains circulating in Botswana. Hozbor DF, editor. PLOS ONE. 2019 May 7;14(5):e0216306.

33. World Health Organization. Definitions and reporting framework for tuberculosis. 2013 revision [Internet]. WHO, Geneva; 2013.

34. Chihota V, Apers L, Mungofa S, Kasongo W, Nyoni IM, Tembwe R, et al. Predominance of a single genotype of Mycobacterium tuberculosis in regions of Southern Africa. Int J Tuberc Lung Dis. 2007;11(3):311–318.

35. Van Der Spuy GD, Kremer K, Ndabambi SL, Beyers N, Dunbar R, Marais BJ, et al. Changing Mycobacterium tuberculosis population highlights clade-specific pathogenic characteristics. Tuberculosis. 2009 Mar;89(2):120–5.

36. Marais BJ. The global tuberculosis situation and the inexorable rise of drug-resistant disease. Adv Drug Deliv Rev. 2016 Jul;102:3–9.

37. Cohen T, Jenkins HE, Lu C, McLaughlin M, Floyd K, Zignol M. On the spread and control of MDR-TB epidemics: An examination of trends in anti-tuberculosis drug resistance surveillance data. Drug Resist Updat. 2014 Oct;17(4–6):105–23.

38. Johnstone-Robertson SP, Mark D, Morrow C, Middelkoop K, Chiswell M, Aquino LDH, et al. Social Mixing Patterns Within a South African Township Community: Implications for Respiratory Disease Transmission and Control. Am J Epidemiol. 2011 Dec 1;174(11):1246–55.

39. Noppert GA, Yang Z, Clarke P, Ye W, Davidson P, Wilson ML. Individual- and neighborhood-level contextual factors are associated with Mycobacterium tuberculosis transmission: genotypic clustering of cases in Michigan, 2004–2012. Ann Epidemiol. 2017 Jun;27(6):371-376.e5.

40. Cooke G, Beaton RK, Lessells R, John L, Ashworth S, Kon O, et al. International Spread of MDR TB from Tugela Ferry, South Africa. Emerg Infect Dis [Internet]. 2011 Nov [cited 2017 Feb 7];17(11).

41. Nava-Aguilera E, López-Vidal Y, Harris E, Morales-Pérez A, Mitchell S, Flores-Moreno M, et al. Clustering of *Mycobacterium tuberculosis* Cases in Acapulco: Spoligotyping and Risk Factors. Clin Dev Immunol. 2011;2011:1–12.

42. Abubakar I, Dara M, Manissero D, Zumla A. Tackling the spread of drug-resistant tuberculosis in Europe. The Lancet. 2012 Jan;379(9813):e21–3.

43. Kornylo-Duonga K, Kima C, Cramera EH, Buff AM, Rodriguez-Howell D, Doyle J, et al. Three air travel-related contact investigations associated with infectious tuberculosis, 2007–2008. Travel Med Infect Dis. 2010 Mar;Volume 8(2):120–8.

44. Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F, et al. Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study. Lancet Infect Dis [Internet]. 2018 Oct [cited 2018 Nov 5];

45. Johnson R, Warren R, Strauss OJ, Jordaan AM, Falmer AA, Beyers N, et al. An outbreak of drug-resistant tuberculosis caused by a Beijing strain in the Western Cape, South Africa. :3.

46. Golesi F, Brignatz J, Bellenfant M, Raoult D, Drancourt M. Mycobacterium tuberculosis Beijing outbreak in a school in Marseille, France, 2012. :2.

47. Schaefer R, Gregson S, Takaruza A, Rhead R, Masoka T, Schur N, et al. Spatial patterns of HIV prevalence and service use in East Zimbabwe: implications for future targeting of interventions. J Int AIDS Soc. 2017;20(1):21409.

48. Timire C, Sandy C, Kumar AMV, Ngwenya M, Murwira B, Takarinda KC, et al. Access to second-line drug susceptibility testing results among patients with Rifampicin resistant tuberculosis after introduction of the Hain Line Probe Assay in Southern provinces, Zimbabwe. Int J Infect Dis. 2019 Apr;81:236–43.

49. Muttamba W, Ssengooba W, Kirenga B, Sekibira R, Walusimbi S, Katamba A, et al. Health seeking behavior among individuals presenting with chronic cough at referral hospitals in Uganda; Missed opportunity for early tuberculosis diagnosis. :9.

50. Ngangro NN, Ngarhounoum D, Ngangro MN, Rangar N, Siriwardana MG, des Fontaines VH, et al. Pulmonary tuberculosis diagnostic delays in Chad: a multicenter, hospital-based survey in Ndjamena and Moundou. BMC Public Health. 2012 Dec;12(1):513.

51. Kompala T, Shenoi SV, Friedland G. Transmission of Tuberculosis in Resource-Limited Settings. Curr HIV/AIDS Rep. 2013 Sep;10(3):264–72.

52. Viegas SO, Machado A, Groenheit R, Ghebremichael S, Pennhag A, Gudo PS, et al. Mycobacterium tuberculosis Beijing Genotype Is Associated with HIV Infection in Mozambique. Gagneux S, editor. PLoS ONE. 2013 Aug 7;8(8):e71999.

53. Chinomona A, Mwambi HG. Estimating HIV Prevalence in Zimbabwe Using Population-Based Survey Data. Tang JW, editor. PLOS ONE. 2015 Dec 1;10(12):e0140896.

54. Gandhi NR, Andrews JR, Brust JCM, Montreuil R, Weissman D, Heo M, et al. Risk factors for mortality among MDR- and XDR-TB patients in a high HIV prevalence setting. Int J Tuberc Lung Dis. 2012 Jan 1;16(1):90–7.

55. Rie AV, Mellet K, John M-A, Scott L, Page-Shipp L, Dansey H, et al. False-positive rifampicin resistance on Xpert® MTB/RIF: case report and clinical implications. 2013;6.

56. Cayci YT, Bilgin K, Coban AY, Birinci A, Durupınar B. An evaluation of false-positive rifampicin resistance on the Xpert MTB/RIF. Mem Inst Oswaldo Cruz. 2017 Nov;112(11):756–9.

57. Mathys V, Van De Vyvere M, Droogh E, Soetaert K, Groenen G. False-positive rifampicin resistance on Xpert® MTB/RIF caused by a silent mutation in the rpo B gene. Int J Tuberc Lung Dis. 2014 Oct 1;18:1255–7.

58. Peilei Hu, Hongtai Zhang, Joy Fleming, Guofeng Zhu, Shuai Zhang, Yaguo Wang, et al. Retrospective Analysis of False-Positive and Disputed Rifampin Resistance Xpert MTB/RIF Assay Results in Clinical Samples from a Referral Hospital in Hunan, China. J Clin Microbiol. Volume 57(4).

59. Lurie MN, Williams BG. Migration and health in Southern Africa: 100 years and still circulating. Health Psychol Behav Med Open Access J. 2014;2(1):34–40.

60. Corno L, De Walque D. Mines, migration and HIV/AIDS in Southern Africa. J Afr Econ. 2012;ejs005.

61. Centis R, D'Ambrosio L, Zumla A, Migliori GB. Shifting from tuberculosis control to elimination: Where are we? What are the variables and limitations? Is it achievable? Int J Infect Dis. 2017 Mar;56:30–3.

62. Pandey P, Pathak H, Dave S. DNA Fingerprinting of Mycobacterium Tuberculosis Isolates from 'Idgah'Agra Region by using IS6110 Probe. [cited 2017 Feb 7];

Table 1 Comparison of sampled to successfully retrieved RR/MDR-TB isolates by region, Zimbabwe, 2011-2016

Variable	Sampled isolates from databases, N=400		Retrieved isolates successfully spoligotyped , N=184		p-value
	North N =141(35.3)	South N = 259 (64.7)	North N = 43 (23.4)	South N = 141 (76.6)	
Mean age (years)	36.65	35.06	36.77	33.78	0.021
Gender					
Male	78 (55.32)	112 (43.24)	27 (62.79)	73 (52.14)	0.220
Female	63 (44.63)	147 (56.76)	16 (37.21)	67 (45.36)	

Table 2 Characteristics of RR/MDR-TB patients in study cohort, Zimbabwe, 2011-2016

Characteristic	Total N=184	Northern N (%)	Southern N (%)	P-value
Patient type	184 (100)			
New	70 (30.0)	26 (60.5)	44 (38.25)	
Retreatment	89 (47.5)	12 (27.9)	77 (55.0)	P<0.001
Missing	25 (13.6)	5 (11.6)	20 (14.2)	
HIV status	162 (100)			
Positive	98 (60.49)	18 (46.15)	80 (65.04)	p= 0.0788
Negative	64 (39.51)	21 (53.85)	43 (34.96)	
Treatment outcome	183	43	141	
Completed	87 (47.3)	1 (2.3)	86 (61.0)	
Cured	32 (17.4)	28 (65.1)	4 (2.8)	P<0.001
Died	28 (15.2)	4 (9.3)	24 (17.0)	p=0.292
Loss to follow up	11 (6.0)	1 (2.3)	10 (7.1)	
Missing	25 (13.6)	8 (18.6)	17 (12.1)	

Table 3 Mean age, gender and treatment outcome of RR/MDR-TB strain, Zimbabwe, 2011-2016

Characteristic	Total	Beijing	LAM11_ ZWE	LAM4	T1	S	Other strains
Mean age	184	35.1±10.4	35.1± 10.8	30.3± 8.1	33.3± 7.5	32.9±11.5	34.5±10.3
Gender	184	45	20	14	25	20	59
Male	100	30 (67)	9 (45)	9 (64)	15 (60)	8 (40)	29 (49)
Female	83	15 (33)	11 (55)	5 (36)	10 (40)	12 (60)	30 (51)
Missing	1	1 (2.2)					
HIV status	184	46	20	14	25	20	59
Positive	98 (54)	28 (62)	10 (50)	7 (50)	14 (56)	11 (55)	28 (47)
Negative	64 (35)	13 (29)	6 (30)	5 (36)	7 (28)	6 (30)	27 (46)
Missing	22 (11)	5(10.9)	4 (20)	2 (14)	4 (16)	3 (15)	4 (7)
Treatment outcome	184	46	20	14	25	20	58
Treatment completion	87 (47)	21 (46)	10 (50)	10 (71)	10 (40)	12 (60)	24 (41)
Cured	32 (17)	6 (13)	5 (25)	0 (0)	6 (24)	0 (0)	15 (26)
Died	28 (15)	9 (20)	1 (5)	2 (14)	2 (8)	2 (10)	10 (17)
LTFU	11 (6)	4 (9)	0 (0)	0 (0)	2 (8)	2 (10)	3 (5)
Missing	25 (14)	6 (13)	4 (20)	2 (14)	4 (16)	4 (20)	6 (10)
Patient type	184	46	20	14	25	20	59
Retreatment	89 (48)	27 (56)	7 (35)	7 (50)	11 (44)	11 (55)	26 (44)
New	70 (38)	13 (29)	9 (45)	5 (36)	10 (40)	6 (30)	27 (46)
Missing	25 (13)	6 (13)	4 (20)	2 (14)	4 (16)	3 (15)	6 (10.2)

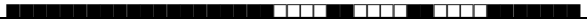
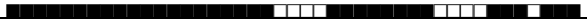
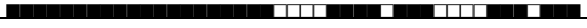
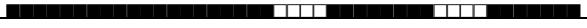
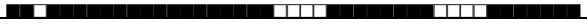
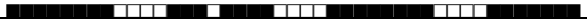
Footnote: LAM_ZWE - Latin American Mediterranean 11_Zimbabwe

LAM – Latin American Mediterranean

Table 4 Data used to draw spatial distribution of RR/MDR-TB in countries neighbouring Zimbabwe, 2011-2016

Family	Zimbabwe		South Africa							Angola	Botswana
	North	South	Gauteng	JHB	Mpumalanga	North West	Limpopo	Western Cape	KZN	Angola	Botswana
Beijing	8 (19)	38 (27)	6 (13)	71 (30)	37 (17)	10 (37)	6 (38)	17 (14)	2 (3)	0 (0)	9 (14)
LAM11_ZWE	7 (16)	13 (9)	1 (2)	0 (0)	7 (3)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	5 (8)
Other LAM	5 (12)	20 (14)	11 (23)	32 (13)	20 (9)	2 (7)	2 (13)	14 (11)	23 (30)	41 (53)	12 (19)
T1	5 (12)	20 (14)	7 (15)	10 (4)	31 (14)	4 (15)	0 (0)	24 (19)	15 (20)	33 (43)	1 (2)
Other T	3 (7)	8 (6)	0 (0)	0 (0)	13 (6)	1 (4)	1 (6)	9 (7)	5 (7)	2 (3)	1 (2)
S	2 (5)	18 (13)	4 (9)	32 (13)	24 (11)	3 (11)	2 (13)	8 (6)	29 (38)	0 (0)	17 (27)
X	2 (5)	3 (2)	3 (6)	20 (8)	7 (3)	0 (0)	1 (6)	19 (15)	0 (0)	0 (0)	11 (18)
EAI	4 (9)	9 (6.4)	9 (19)	37 (16)	49 (22)	2 (7)	2 (13)	0 (0)	0 (0)	0 (0)	7 (11)
Other	3 (7)	8 (6)	3 (6.4)	37 (16)	15 (7)	2 (7)	0 (0)	12 (10)	2 (3)	1 (1)	0 (0)
Unknown	4 (9)	4 (3)	3 (6)	0 (0)	21 (9)	2 (7)	2 (13)	23 (18)	0 (0)	0 (0)	0 (0)
Total	43	141	47	239	224	27	16	126	76	77	63

Table 5 Spoligotype genotypic distribution of RR/MDR-TB strains showing proportion with phenotypic resistance circulating in Zimbabwe, 2011-2016

CLADE	Family	Spoligotype Pattern	# of isolates	% clustered	Phenotypic DST Pattern			
					RIF	INH	E	STR
Beijing	Beijing		45	25	41	45	45	44
CAS	CAS1_KILI		3	1.6	2	2	2	2
EAI	EAI1_SOM		13	7.1	12	12	12	12
H	H1		5	2.7	4	4	4	4
LAM	LAM11_ZWE		20	10.9	18	18	18	18
	LAM4		14	7.6	13	13	13	13
	LAM6		5	2.7	5	5	5	5
	LAM9		3	1.6	2	2	2	2
	LAM1		1	0.5	1	1	1	1
	LAM3		2	1.0	2	2	2	2
MANU	MANU2		2	1.1	2	2	2	2
S	S		20	8.7	20	20	20	20
T	T1		25	11.4	24	24	24	24
	Other T		11	2.7	9	9	9	9
U	U		1	0.5	1	1	1	1
X	X2/X3		5	2.7	5	5	5	5
unknown			**8**		6	6	6	6
Total			**184**					

Footnote: 1. RIF= rifampicin, INH = Isoniazid, E = Ethambutol and STR = Streptomycin

2. LAM_ZWE - Latin American Mediterranean 11_Zimbabwe

3. LAM – Latin American Mediterranean

Table 6 Distribution of RR/MDR-TB strains showing increased proportion of strains over two time points, Zimbabwe 2011-2016

Family	**Total**	**2011-2012**			**2015-2016**			**P-value**
		Northern	Southern	**Total N (%)**	Northern	Southern	**Total N (%)**	
BEIJING	45	0	18	18 (40)	8	19	27 (60)	<0.001
LAM11_ZWE	20	1	5	6 (30)	6	8	14 (70)	<0.001
Other LAM	25	0	9	9 (36)	5	11	16 (64)	0.003
T1	25	0	11	11 (44)	5	9	14 (56)	0.0811
Other T family	11	0	3	3 (27)	3	5	8 (73)	<0.001
S	20	0	8	8 (40)	2	10	12 (60)	0.206
CAS1_KILI	3	0	1	1 (33)	2	0	2 (67)	
EAI1_SOM	13	0	3	3 (23)	4	6	10 (77)	
U	1	0	1	1 (100)	0	0	0 (0)	
MANU2	2	0	0	0 (0)	0	2	2 (100)	
X2	5	0	1	1 (20)	1	2	4 (80)	
H1/H3	5	0	0	0 (0)	1	4	5 (100)	
Unknown	8	0	0	0 (0)	4	4	8 (100)	
Total	183	1	60	61 (33)	43	80	122 (67)	

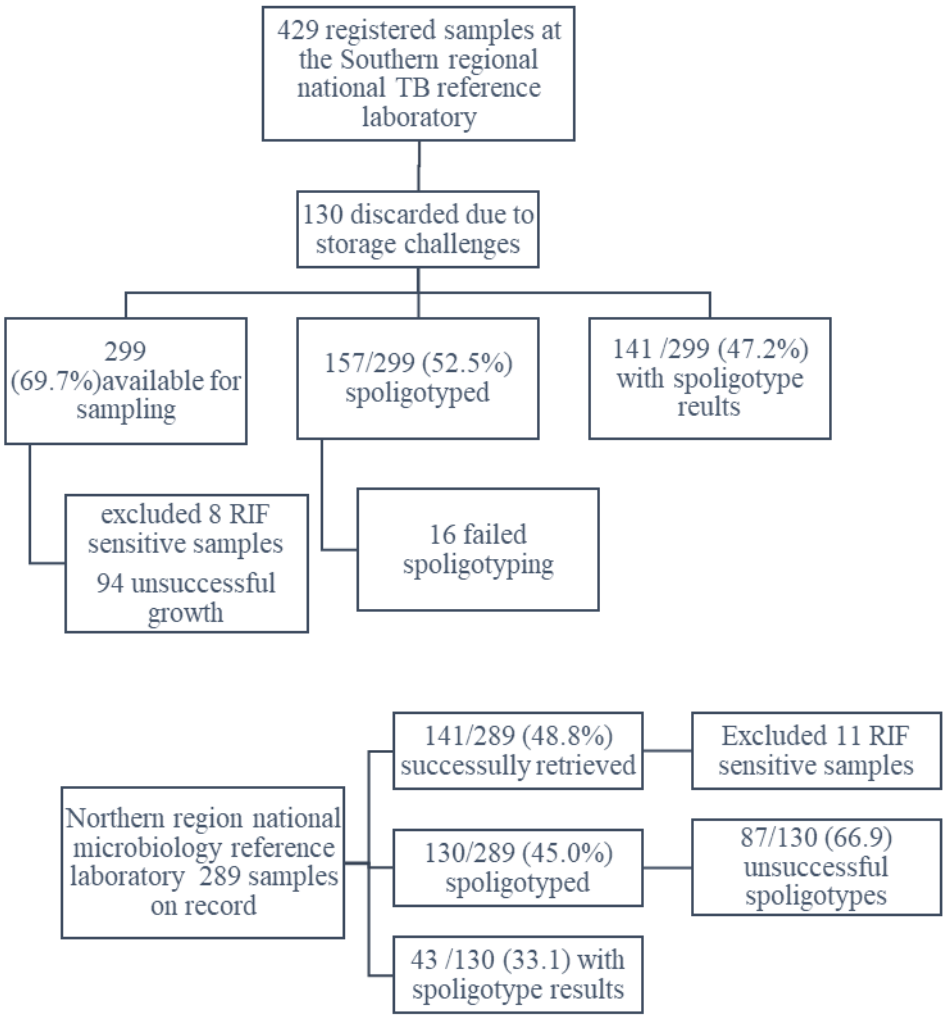


Figure 1 RR/MDR-TB sample selection from the NMRL and NTRL, Zimbabwe 2011-2016

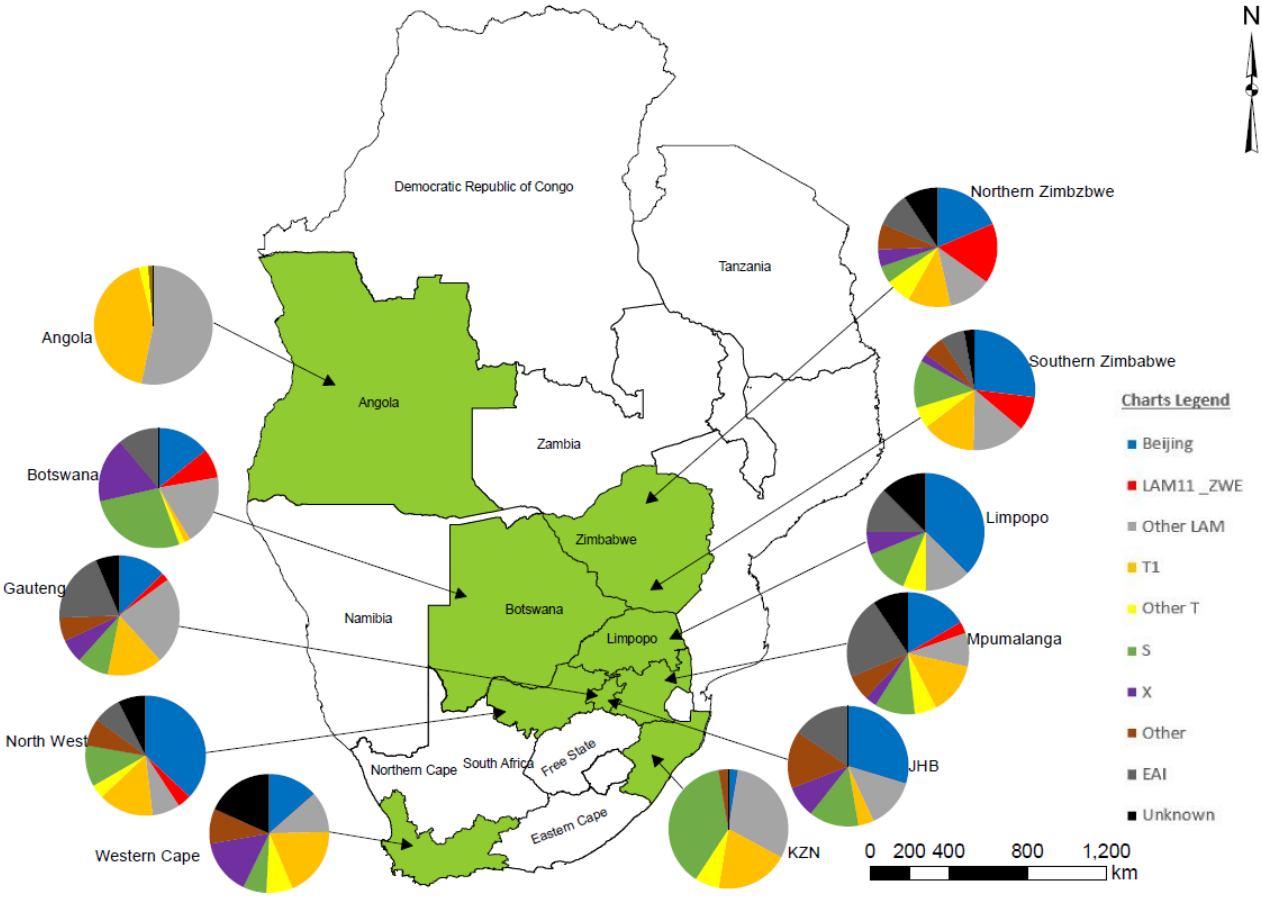


Figure 2 Genetic diversity of DR-TB strains of countries neighbouring Zimbabwe, 2011-2016

Chapter 7

Phylogeography of drug resistant Lineage 2 strains in Zimbabwe and South Africa

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This chapter describes the spread of drug resistant tuberculosis (DR-TB) lineage 2 (L2) between South Africa and Zimbabwe using publicly available whole genome sequencing (WGS) South African data, data from the northern and southern DR-TB isolates and isolates from Harare city.

My contribution was design, part of laboratory work for the northern and southern region isolates, writing the initial draft manuscript and analysis to produce maps and phylogenetic trees using nextstrain and interactive tree of life (ITOL).

Phylogeography of drug resistant Lineage 2 strains in Zimbabwe and South Africa

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Abstract

Background: Higher mortality among susceptible tuberculosis (TB) patients from Zimbabwe's southern region has been attributed to high human immunodeficiency virus (HIV) infection, being a retreatment case and age above 65 years old. Increasing presence of rifampicin resistant (RR) TB Lineage 2 (L2), T and S strains in Zimbabwe maybe an indication of changing RR/MDR-TB epidemiology affecting TB treatment outcomes.

Objective: We analysed available whole genome sequence data of drug resistant *Mycobacterium tuberculosis* (*Mtb*) strains from Zimbabwe and South Africa to describe the movement of L2 DR-TB strains between the two countries.

Methods: *Mtb* genome read data deposited in the National Center for Biotechnology, United States National Library of Medicines Sequence Read Archive (NCBI SRA), for Southern Africa were downloaded. These sequence reads were analysed together with sequence reads from *Mtb* isolates cultured from patients diagnosed with drug resistant TB in Zimbabwe between 2010 and 2016. We used Nextstrain and Interactive Tree of Life (ITOL) bioinformatics software to describe temporal clustering, drug resistance patterns and directional movement of the L2 strain between Zimbabwe and South Africa.

Results: Two lineages, L2 and L4, were predominant in both Zimbabwe and South Africa, with L1 and L3 exclusively common in Zimbabwe and South Africa, respectively. Phylogeography tree and maps showed that there was bidirectional spread of ancestral L2 strains. Despite the presence of temporal clustering, the genotypic resistance profiles were different. South Africa had more pre-extremely and extremely drug resistant TB (Pre-XDR and XDR-TB) and Zimbabwe had more multidrug resistant tuberculosis (MDR-TB). Drug resistance-conferring genotypic markers for Zimbabwean isolates showed that pyrazinamide resistance in combination with streptomycin was relatively high, 16.7% with low fluoroquinolone resistance, 1.7%. Amino acid changes in genes linked to bedaquiline (BDQ) and delamanid resistance were present in the isolates from Zimbabwe.

Conclusion: Spread of DR-TB L2 strains between South Africa and Zimbabwe could not be categorically demonstrated. Presence of BDQ and delamanid resistance markers in Zimbabwean isolates may complicate the introduction of proposed new BDQ based new regimens for the treatment of RR-TB. Strengthening of national TB programmes through the development of a Southern African Development Community (SADC) cross border RR/MDR-TB transmission research study is strongly recommended.

7.1 Introduction

Despite the global decline in the *Mycobacterium tuberculosis* (*Mtb*) incidence, the burden of rifampicin/multidrug resistant tuberculosis (RR/MDR-TB), threatens the objectives of the End TB targets (1). The true costs of treating RR/MDR-TB in low income countries are largely unknown. However Africa, home to nine (9) high triple burden of TB, MDR-TB and human immunodeficiency virus (HIV) could be paying relatively more to treat RR/MDR-TB (2,3). The SADC region is known for the high generalised HIV prevalence, free movement of citizens and poverty (4,5). Poverty, over-crowding and inadequate health infrastructure for early diagnosis and treatment, propagate the transmission of MDR-TB in low income countries (6–8).

Although minimal evidence is available to confirm the contribution of migration to the spread of MDR-TB, the current global distribution of *Mtb* lineages is believed to have been shaped by migration (9–16). As discussed in Chapter 6, migration and spread of RR/MDR-TB remains an area of research need especially in high TB burden settings where research has been minimal. The discovery of gold in the early 20th century and policy on free movement of labour between SADC countries, promotes increased cross-border movement with a net population gain in countries with stronger economies (13,14). The Lineage 2 (L2) strains have been implicated in MDR-TB outbreaks in low and high TB burden countries due to their virulence and transmissibility especially among HIV positive populations (18–22). The drug resistant (DR)-TB L2 strain population was shown to be common in South Africa's Limpopo,

Gauteng, and Western Cape provinces with L4 strain being more common in Botswana (23,24). Previously, we reported an increased prevalence of MDR-TB cases around the southern region of Zimbabwe, Limpopo and Gauteng provinces and Botswana. We hypothesized that long term migration patterns between Zimbabwe and South Africa may have resulted in increased cross-border transmission of the L2 strains and that this was responsible for the high TB mortality in Zimbabwe's southern region (16, 22). In this study we test this hypothesis using whole genome sequencing of drug resistant clinical isolates cultured from patients attending clinics in Zimbabwe to determine genetic similarity between L2 strains from both countries as a proxy for cross-border transmission.

7.2 Materials and Methods

7.2.1 Study Setting

Since the inception of PMDT in 2010 to 2016, the Zimbabwe National Tuberculosis and Leprosy Control Programme (NTP) had enrolled an estimated 2,158 RR/MDR-TB cases, an under estimation according to the WHO (1). The public health infrastructure related to the management of DR-TB has been inadequate in Zimbabwe, with only 51% of the MDR-TB patients having access to culture and drug sensitivity testing (CDST) services (27). The low access to CDST in Zimbabwe may have resulted in delayed diagnosis and treatment of RR/MDR-TB patients, with it, increasing the likelihood of a sustained MDR-TB outbreak (28). Zimbabwe used a 24 months standardised RR/MDR-TB treatment regimen of 6 months Pyrazinamide (PZA), kanamycin (Km), Levofloxacin (Lfx), Cycloserine (Cs), Prothionamide (Pto), para-amino-salycilic acid (PAS) and 18 months of Lfx, Cs, Pto, PAS from 2011 to 2018 when the country adopted the shorter treatment regimen of 4-6 months (Km, Moxifloxacin (Mfx)^{HD}, Ethionamide (Eto), Clofazimin (Cfz), Isoniazid (INH)^{HD}, PZA, Ethambutol (E) /5 months of Mfx, Cfz, PZA, E.

South Africa (SA) is one of the high MDR-TB burden countries with 3.4% of new notifications and 7.1% of retreatment estimated to be MDR-TB cases in 2017 (1). In 2017, South Africa initiated 11,192/19,073 (58.7%) RR/MDR-TB patients on treatment and 56.6% of all diagnosed MDR-TB cases had second line DST done (2). Although South Africa required additional expansion of the laboratory capacity around 2017-2018, the country had well organized TB laboratory network to offer first and second line DST compared to other African countries (29). The standardized treatment regimen for South Africa from 2009 until 2018 had 6 months of Km, INH^{HD}, EtoMfx, Cfz, PZA, E followed by 18 months of, Mfx, Cfz, PZA, E. At the end of 2018, South Africa introduced the shorter all oral regimen of 6 months of Bedaquiline (BDQ), high dose INH, Lfx, Cfz, PZA and E followed by 5 months of Lfx, Cfz, PZA and E (30)

7.2.2 Study Population

Genome read data in National Center for Biotechnology, United States National Library of Medicine Sequence Read Archive (NCBI SRA) were queried for BioSamples with metadata identifying the species as *Mycobacterium tuberculosis* (*Mtb*) and a geographic location name in Southern Africa (31). A total of 5553 files fitting these criteria were downloaded. Genome sequence data from in-house sequenced isolates collected in Zimbabwe were added to the set (n=311). Only samples that contained data, aligned to H37Rv with significant breadth of coverage (>94% of H37Rv covered at 10X depth), and passed SNP calling were included in further analyses (n=5437 BioSamples, n=311 in-house genomes). Isolates from South Africa had been collected as far back as 2009 through to 2016. Isolates from Zimbabwe were from 2011 to 2016.

7.2.3 *Mtb* strain typing

Mtb lineages were determined using canonical nucleotide positions identified from a single nucleotide polymorphism- (SNP-) based phylogenetic tree of *Mtb* (32). For this specific

phylogenetic analysis, genome assemblies from the NCBI Assembly database, and sequence read data from Bioprojects PRJEB9201, PRJEB3124, and PRJEB3163 in the NCBI SRA were downloaded (33). SNP matrices to identify point mutations among the isolates (and thus infer strain relatedness) were generated with NASP, in which reads were aligned to the publicly available H37Rv genome (Genbank accession no. NC_000962.3) using BWA (34). SNPs were called with genomic analysis toolkit (GATK), and were only included in further analyses if they were a) present in all reads, b) covered by ≥10X depth with ≥90% consensus in each sample, and c) not in any duplicated regions in the reference genome as identified by NUCmer (35,36). The resulting SNP matrix comprised the core genome common to all samples in the analysis. Maximum parsimony analyses were performed with MEGA v7.0 (37).

Canonical SNPs distinguishing Lineages 1 to 7 were identified using this resulting phylogenetic tree and the NASP SNP matrix. The SNP loci and flanking sequence for three canonical SNPs for each lineage were used as reference sequences for genotyping the southern Africa genomes.

Genotypes of the canonical SNP loci of each of the southern Africa genomes were determined using amplification, sequencing and annotation of plastomes (ASAP) (38,39). ASAP uses a JavaScript Object Notation (JSON) file to define each SNP locus, the strain type of each genotype, and the reference sequences for read mapping. In ASAP, sequence reads mapped to the reference sequences with BWA (40) (35). Resulting BAM alignment files were analyzed alongside the JSON file definitions to determine each sample's genotype. Genomes successfully genotyped were binned for phylogenetic analysis based on their lineage (n=5118 BioSamples, n=285 in-house genomes, n=334 Lineage 1, n=1103 Lineage 2, n=337 Lineage 3, n=3536 Lineage 4, n=6 Lineage 6, n=87 mixed lineages).

7.2.4 Phylogenetic analysis

A subset of lineage 2 Biosamples from Zimbabwe and South Africa was analysed to assess presence of transmission between the two countries. With the filtered sample sets (all genomes that passed initial NASP filters, see above), NASP was used to generate SNP matrices for lineage 2. Maximum likelihood phylogenetic trees for lineage 2 strains from South Africa and Zimbabwe were generated using iq-tree with the GTR model (40)

7.2.5 NextStrain

Configuration files composed for NextStrain for visualization and filtering purposes included ASAP results for lineage 2, location and collection data of each isolate and drug resistance genotypes as highlighted in Miotto et al. (41). Lineage 2 strain phylogenetic trees were uploaded into NextStrain and scaled to time using TimeTree (41,42). Configuration files were uploaded, and analyses run in NextStrain. Determination of estimated dates of divergence were ascertained by viewing all relevant information displayed on the branch or isolate on the phylogenetic tree

7.2.6 Ethical Clearance

This study was approved by the institutional review boards for University of Stellenbosch University, approval number, S16/06/106, and the Medical Research Council of Zimbabwe (MRCZ), approval number, MRCZ/A/1830 and the University of California, San Francisco, approval number, 10-05115.

7.3 Results

7.3.1 Resistance patterns of all DR-TB strains between South Africa and Zimbabwe

Publicly available WGS data from South Africa included 670 (77.2%) and Zimbabwe had 198 (23.0%) strain sequences. The L2 (Beijing genotype strain) and L3 were the predominant strains in South Africa contributing about 78.8%, (528/670) and 5.1%, (34/670) of the total isolates from South Africa respectively (Figure 1 and Table 1). In Zimbabwe the predominant strains were the L1, 9.1% (18/198) and L4, 58.1% (115/198) of the total Zimbabwean strain population.

7.3.2 Resistance patterns of L2 DR-TB strains in South Africa and Zimbabwe

Analysis of L2 sequences from both countries showed that the lineages were related through shared ancestral nodes (Figure 1). However, their genotypic drug resistance markers were different, with South African strains having a higher representation of MDR-TB and pre-XDR-TB (Table 2). The most common drug resistance was Streptomycin mono-resistant, 359 (59.3%), followed by rifampicin and streptomycin polyresistant TB, 38 (6.5%) and multidrug resistant (MDR) TB in combination with streptomycin resistance, 36 (6.1%). A total of 80 (13.6%) were extremely drug resistant (XDR) TB from South Africa compared to 5 (0.8%) in Zimbabwe. The combined total pre-XDR-TB was 53 (9.0%). There were 41 (7.0%) any rifampicin (RIF) resistance and 16 (2.7%) any isoniazid (INH) drug resistance strains.

7.3.3 Temporal clustering and drug resistance profile of Lineage 2 strains in Zimbabwe and South Africa

Temporal clustering referred to time of diagnosis and treatment of the isolate source patient. Figures 2 shows temporal clustering of the L2 strains from South Africa and Zimbabwe. The blue (10 o'clock) and brown (11 o'clock) position clusters show cases from Zimbabwe diagnosed between 2012 and 2016 and those diagnosed in South Africa between 2009 and 2015. However, resistance patterns were different, with the South African isolates showing

additional resistance to injectables and quinolones (Supplemental Figure (SF) 2a-b). In contrast the majority of Zimbabwean isolates had pyrazinamide resistance and were all from the southern part of the country. In the purple (4 o'clock) position cluster all isolates from Zimbabwe were MDR with pyrazinamide resistance compared to the South African strains that were predominantly XDR. Both sets of isolates had streptomycin resistance (SF 2b). The green cluster showed some L2 strains that were closely related between the northern and southern regions of Zimbabwe and were all MDR (TB-CPath-Midori-IS-1036-56-57 and TB-CPath-Joconiah-IS-1028-TMS-850) (SF 2b). All South African L2 strains within the cluster were rifampicin and streptomycin poly-resistant. In supplementary figure 2f (SF 2f), we included the branch length to demonstrate the relatedness of the strains between South African and Zimbabwean strains. Results of temporal clustering showed that patients sharing common ancestor were diagnosed and treatment around the same time period irrespective of country of origin

7.3.4 Directional movement of DR-TB L2 strains, Zimbabwe and South Africa

The directional phylogenetic tree estimated an earlier presence of the L2 strain in South Africa before the spread to the North of the country (Figure 3). Spread of L2 to Zimbabwe may have started around the early 1900s (Figure 3 and Figure 6). During this early presence of the L2 strain in South Africa and northward spread, there was presence of amino acid changes in the common ancestor, namely, the PPE20 (E57D), Rv0323c (S142G), Rv1218c (Q243R), cyp141 (K157E), Rv1520 (R251C) and Rv2771c (L80P). The map showed that the movement of L2 strains was mainly from South Africa to Zimbabwe (purple lines originating from the large circles in South Africa), subsequent spread was from either of the country (Figure 4-6). Common amino acid changes in the L2 strains circulating in Zimbabwe during this early period of movement were Rv1144 (S11T), ATP synthetase subunit c (*atpE)* (G362A), *pabC* (A107V), *pcnA* (S394A). The map showing spread by location suggests that spread of the L2 strains was more common in the Southern parts of Zimbabwe (Figure 4

and 5). From the available sequence data, it appeared as if two provinces in South Africa were source provinces, namely Eastern Cape and Kwazulu-Natal (Figure 5). However, this may have been influenced by the availability of sequence data and must be interpreted with caution. As early as the late 1990s, there were L2 strains that had variants in the *fbiA* (I208V) and *atpE* (G362A) genes that are implicated in resistance to Delamanid and Bedaquiline.

7.4 Discussion

7.4.1 Predominant DR-TB lineages in South Africa and Zimbabwe

Our results showed that L4 was the most predominant strain lineage in Zimbabwe whilst L2 strains were more common in the available South African *M. tuberculosis* sequences. Previous studies have confirmed the predominance of L4 in Zimbabwe and L2 in some provinces of South Africa (43,44). The results also showed that there was bidirectional spread, L2 strains from South Africa to Zimbabwe and from Zimbabwe to South Africa. The apparent exclusive presence of L1 and L3 in Zimbabwe and South Africa and why these strains have not been able to spread between the two countries are areas of research needs.

7.4.2 Increased population of DR-TB L2 strains in South Africa and Zimbabwe

Our results show that L2 strains remained dominant and continued to grow in both South Africa and Zimbabwe. There are a few possible reasons for this observation. First, the L2 strain is known to be more virulent, occur in epidemic form, affecting the highly mobile young male population and those with HIV infection (45). Both Zimbabwe and South Africa have a generalized HIV epidemic that has fuelled the TB epidemic (4). Secondly, this may not be a true increase in the L2 population, but just a result of improved TB diagnostic capacity using new technologies that has allowed proper characterisation of circulating MDR-TB strains (46). The third reason could be the improved capacity to conduct scientific research on DR-TB than other countries in the region. A combination of high DR-TB burden, available

funding and human resource capacity in South Africa has facilitated the conduct of more research studies on MDR-TB using molecular technologies. The fourth possible reason is related to the inadequate access to DR-TB diagnosis and treatment capacity in Zimbabwe where only 40% of all presumptive TB cases had access to second line CDST (27). In the absence of diagnostic capacity, infectious persons with DR-TB will infect more persons before being diagnosed (45). Combined with the virulence and easy transmissibility, low access to TB diagnostic services maybe the most plausible reason for the expansion of the DR-TB L2 strain in Zimbabwe (47). Fifth, because of high TB transmission between the two countries, there were possibilities of one individual patient having dual infections, with both susceptible and DR-TB L2 strains (48).

7.4.3 Is migration the possible cause of the increased RR/MDR-TB L2 population in Zimbabwe?

Although our results did not categorically demonstrate presence of cross border spread of L2 from South Africa to Zimbabwe, the following reasons may suggest that the hypothesis was due to migration. An epidemiological link exists that support the expansion of L2 into Zimbabwe. First, the expansion seemed to have been preceded by the first documented movement of labour to work in gold mines (22,49). Second, previous studies have confirmed the spread of TB from South African mining towns to rural South Africa and beyond during the 1800 period (17). Third, the spread of L2 from migration is well documented in Europe, where the social instability in the former Soviet Union was cited as the main driver of L2 spread (50). Fourth, because of the high triple burden of TB, TB/HIV and RR/MDR-TB in Zimbabwe and South Africa, there were possibilities of dual infection by two different strains, one susceptible and the other resistant (48). We therefore recommend strengthening of the public health system to allow early diagnosis and treatment of all MDR-TB cases to interrupt transmission.

7.4.4 Implications of pre-existing DR-TB to re-purposed medicines, BDQ and Delamanid

Of note was the presence of variants in genes associated with Delamanid and BDQ resistance, *fbiA* and *atpE* in Zimbabwe. The new World Health Organization (WHO) recommended short MDR-TB treatment regimens include BDQ and Delamanid (51). Resistance to BDQ has already been reported in clinical settings including some studies from South Africa (52–54). Mechanism of BDQ resistance include spontaneous mutations, cross resistance with clofazimine and drug pressure from inadequate treatment (55). However, in this study, the estimated time of BDQ resistance indicate that the drug had not yet been introduced as part of the treatment regimen. Spontaneous mutation of the Rv0678 may explain the observed BDQ resistance pattern in these isolates.

Several challenges associated with the introduction of the new WHO recommended shorter MDR-TB regimen exist. First, it would be important to understand fully the mechanism of BDQ resistance in Zimbabwe. One study suggests that inadequate treatment from weak TB programmes that results in poor adherence and inadequate monitoring of drug susceptibility was promoting development of BDQ resistance through causing drug pressure (52). Two studies have reported acquired resistance to BDQ and one study suggested that there could be pre-existing BDQ resistance in populations from high MDR-TB burden(52,56,57). Our results also showed that there were mutations in BDQ resistance conferring genes well before the drug was introduced as part of the regimen in the African setting. A study to estimate pre-existing BDQ resistance in populations with high MDR-TB burden is an urgent requirement. Secondly, there was a strong recommendation to build capacity for BDQ DST and genetic based resistance testing during use of the drug (53). In high MDR-TB burden countries, where the use of BDQ is required most, there are existing weak laboratory capacity. Balancing the need to use BDQ without promoting development of its resistance and building capacity for second line DST would be a major challenge.

There is therefore need for continuous anti-TB drug development to mitigate the challenges of drug resistance.

7.4.5 Resistance profile among DR-TB L2 strains in Zimbabwe

Our results showed that the DR-TB L2 strains in Zimbabwe had low levels of fluoroquinolone resistance but relatively high pyrazinamide resistance. The results also showed that the common genetic ancestor had several amino acid changes, most of the proteins known as *Mtb* growth promoters and a few with changes conferring drug resistance (Figure 3d) (58). This variation in resistance patterns could have been due to a few reasons. First, there may be spread of susceptible or rifampicin monoresistance TB L2 strain from South Africa which is then potentiated to MDR-TB by late diagnosis and inadequate treatment (27). Second, because the region is high burden and possibilities of mixed infection with a sensitive strain in addition to DR-TB were high(48)

The presence of increased levels of PZA resistance in the Zimbabwean isolates may have been due to the previously reported high prevalence of PZA resistance in South Africa's MDR-TB patients (59). PZA resistance is mainly due to mutations in the *pncA*, *rpsA, panD* and efflux pump *Rv1258c*. The actual drivers of these PZA resistance mutations are not known (60). The few DR-TB L2 cases from Zimbabwe with PZA resistance, 10/60 (16.7%), made it difficult to adequately characterise the extent of PZA resistance in relationship to movement of DR-TB L2 strains from South Africa. This will require more analysis with more WGS isolates from Zimbabwe. The implication for the high PZA for the MDR-TB regimen in Zimbabwe could be minimal since current evidence show that even in the presence of very high levels of PZA resistance, its inclusion in the MDR-TB regimen improves treatment success (61). The implications of PZA resistance maybe challenging for the treatment of susceptible TB in Zimbabwe since only three effective drugs will be in use during the intensive phase. This may potentiate the development of MDR-TB from pressure on the rifampicin and isoniazid. However, operational research with the new proposed all oral

regimen where Moxifloxacin, clofazimine, PZA and ethambutol will be used in the continuation phase to monitor PZA resistance using WGS will be required.

The low levels of fluoroquinolone (FQ) resistance among the DR-TB L2 isolates from Zimbabwe was encouraging. The proposed new shorter regimen for Zimbabwe will have levofloxacin and clofazimine as the only potent drugs in the continuation phase. Efforts to preserve the efficacy of FQ through rational use are required to preserve their efficacy (62). In Zimbabwe, first generation FQ are widely used to treat other respiratory tract infections and diarrheal diseases. Recently, evidence of multidrug resistant Salmonella *typhi* to ciprofloxacin in Zimbabwe, South Africa, Zambia and Tanzania could be a sign that more controlled use of FQ is urgent as there tends to be cross resistance (63).

7.4.6 Limitations of the study

There were few to no WGS results from Limpopo and Mpumalanga provinces, which border with Zimbabwe's southern and eastern provinces. This data would have provided more information on the extent of spread of the DR-TB L2. The sample size from Zimbabwe was low, about 10% of the total sample size. This made it difficult to adequately estimate the true burden of DR-TB L2 in the country and the relationship with the South African epidemic. The programmatic management of drug resistant TB (PMDT) in Zimbabwe started recently, in 2010, therefore enrolment was still low, and few research studies of DR-TB had been done. In addition, the short time period of data availability, 2009 to 2016 may have affected the accuracy dating the mutations and estimating the timing of the spread of L2 in our study(41). Despite these limitations and the inability to categorically show presence of MDR-TB spread due to migration between Zimbabwe and South Africa, the available evidence from this study provided important information for designing sound DR-TB prevention and control measures in Zimbabwe.

The South African and Zimbabwean NTP must develop innovative methods to improve cross border MDR-TB prevention and control measures along the European Union regional DR-TB surveillance programme (64). These activities must include surveillance, operational research and new drug development as a minimum. Secondly, a more comprehensive bioinformatic epidemiological study to include drug susceptible TB is recommended for the whole SADC. This will provide more information on the extent of the contribution of migration to the TB epidemic in the SADC region.

Table 1. Distribution of all MDR-TB strains circulating in South Africa and Zimbabwe

	Unknown		**L1**		**L2**		**L3**		**L4**		**Total**	
	N	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
South Africa	25	3.7	2	0.3	528	78.8	34	5.1	81	12.1	670	77.2
Zimbabwe	2	1.0	18	9.1	60	30.3	3	1.5	115	58.1	198	23.0
Total	27	3.1	20	2.3	588	67.7	37	4.3	196	22.6	868	100

Table 2. Resistance profiles of DR-TB L2 strains circulating in South Africa and Zimbabwe based on genotypic drug resistance causing markers

Country	South Africa					Zimbabwe		Grand Total
Region/ province	KwaZulu Natal	Gauteng	Eastern Cape	Western Cape	Unknown	Northern	Southern	N (%)
South Africa	**344**	**4**	**2**	**8**	**171**			**529 (89.8)**
Amikacin-Capreomycin-Isoniazid-Kanamycin-Pyrazinamide-Quinolones-Rifampin-Streptomycin					15			15 (2.5)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Pyrazinamide-Quinolones-Streptomycin					8			8 (1.4)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Pyrazinamide-Rifampin-Streptomycin					1			1 (0.2)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Pyrazinamide-Streptomycin					3			3 (0.5)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Quinolones-Rifampin-Streptomycin			2		19			21 (3.6)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Quinolones-Streptomycin					24			24 (4.1)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Rifampin-Streptomycin	3				5			8 (1.4)
Amikacin-Capreomycin-Isoniazid-Kanamycin-Streptomycin					11			11 (1.9)
Amikacin-Capreomycin-Kanamycin-Pyrazinamide-Quinolones-Streptomycin					1			1 (0.2)
Amikacin-Capreomycin-Kanamycin-Quinolones-Rifampin-Streptomycin					1			1 (0.2)
Amikacin-Capreomycin-Kanamycin-Quinolones-Streptomycin					9			9 (1.5)
Amikacin-Capreomycin-Kanamycin-Rifampin-Streptomycin	1							1 (0.2)

Amikacin-Capreomycin-Kanamycin-Streptomycin	1				16			17 (2.9)
Bedaquiline-Isoniazid-Quinolones-Streptomycin					2			2 (0.3)
Bedaquiline-Quinolones-Streptomycin					1			1 (0.2)
Isoniazid-Kanamycin-Quinolones-Streptomycin					1			1 (0.2)
Isoniazid-Pyrazinamide-Quinolones-Rifampin-Streptomycin		1			1			2 (0.3)
Isoniazid-Pyrazinamide-Rifampin-Streptomycin	2							2 (0.3)
Isoniazid-Quinolones-Rifampin-Streptomycin					2			2 (0.3)
Isoniazid-Quinolones-Streptomycin					1			1 (0.3)
Isoniazid-Rifampin-Streptomycin	6	2			2			10 (1.7)
Isoniazid-Streptomycin	1				3			4 (0.7)
Kanamycin-Streptomycin					1			1 (0.2)
Quinolones					1			1 (0.2)
Quinolones-Streptomycin					1			1 (0.2)
Rifampin	2							2 (0.2)
Rifampin-Streptomycin	29							29 (4.9)
Streptomycin	299	1		5	44			349 (59.3)
Zimbabwe						**19**	**36**	**60 (10.2)**
Amikacin-Capreomycin-Isoniazid-Kanamycin-Quinolones-Rifampin-Streptomycin						1	4	5 (0.8)
Isoniazid-Pyrazinamide-Rifampin-Streptomycin						1	9	10 (1.7)
Isoniazid-Quinolones-Rifampin-Streptomycin						1		1 (0.2)
Isoniazid-Rifampin-Streptomycin						6	18	24 (4.1)
Isoniazid-Streptomycin							1	1 (0.2)
Rifampin-Streptomycin						3	6	9 (1.5)
Streptomycin						7	3	10 (1.7)
Grand Total	344	4	2	8	171	19	36	589 (100)

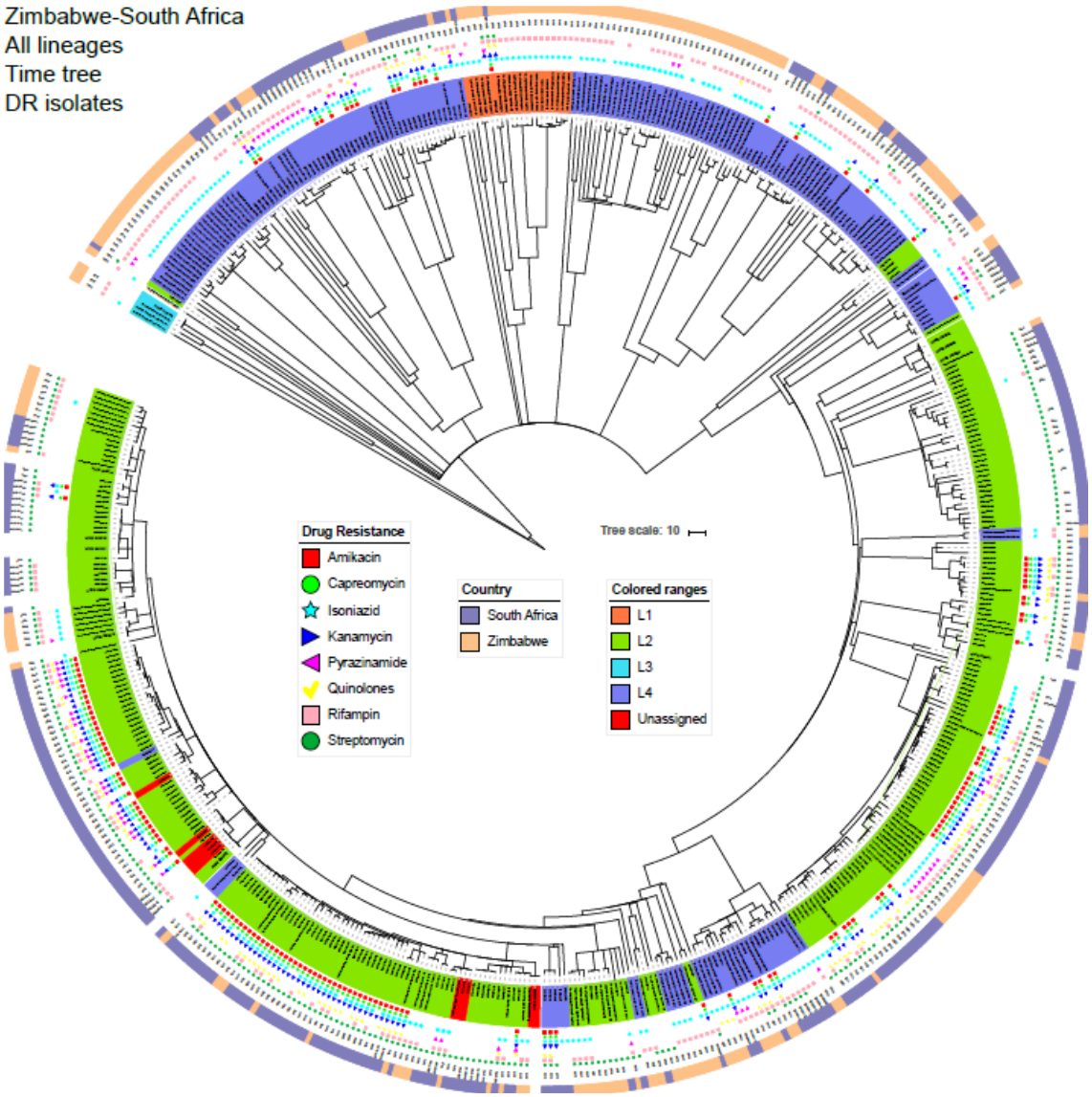


Figure.1 Phylogenetic tree showing the distribution of DR-TB strains by lineage, resistance patterns and country, Zimbabwe and South Africa. *Displayed by screenshot of Interactive Tree of Life (ITOL). https://itol.embl.de/shared/corburn*

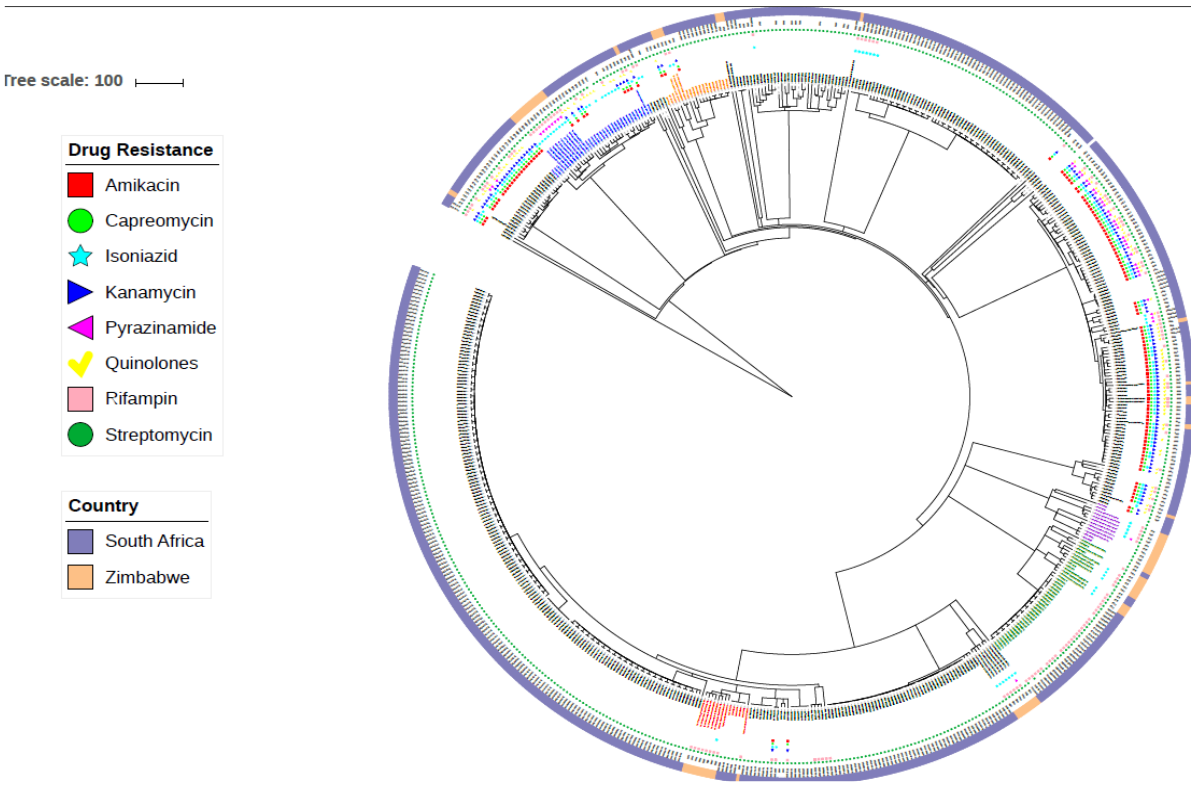


Figure 2. Phylogenetic tree showing the temporal clustering of DR-TB L2 strains, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life (ITOL) software. *https://itol.embl.de/shared/corburn*

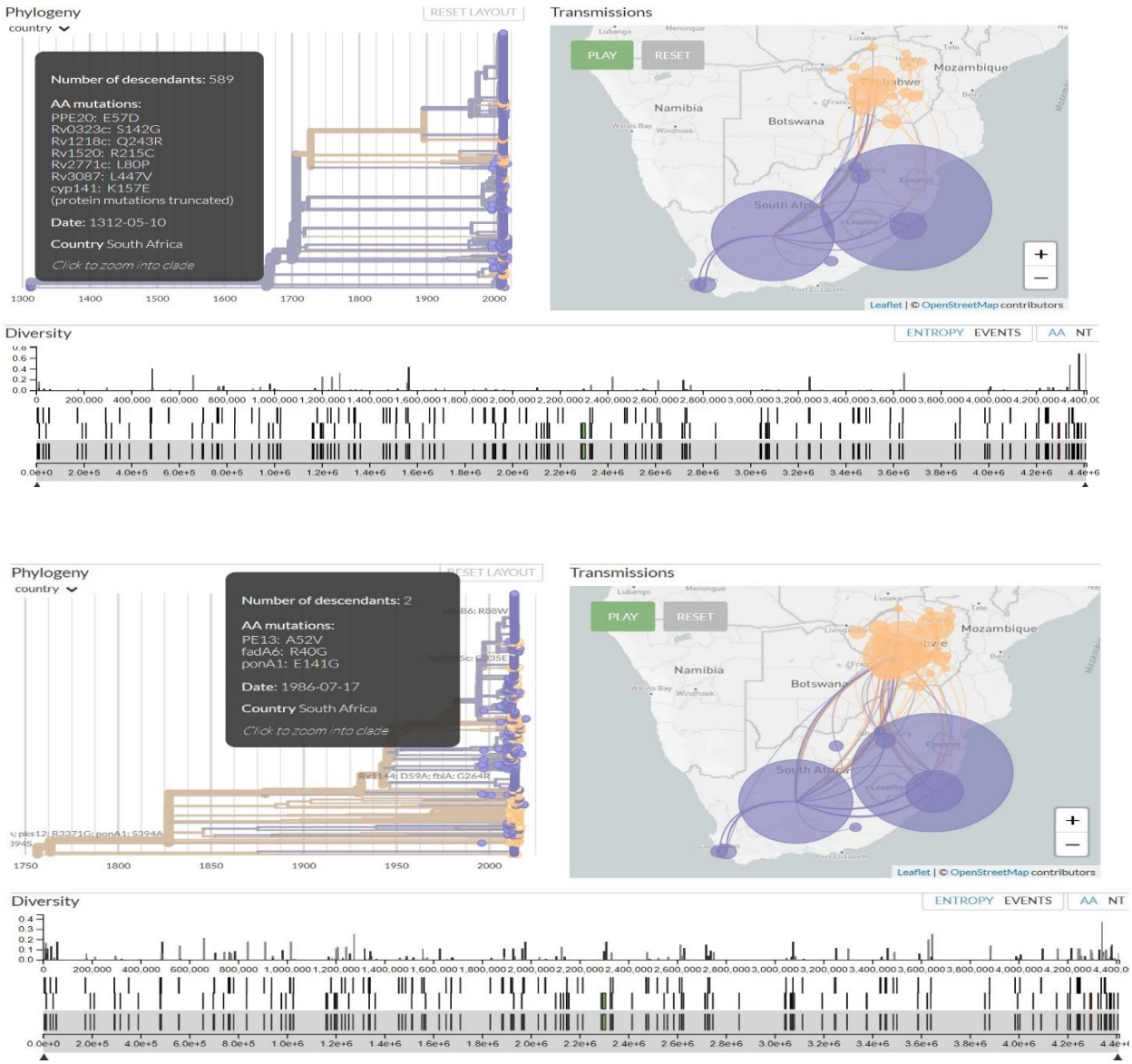


Figure.3 Directional movement of DR L2 strain between Zimbabwe and South Africa, showing common amino acid changes, 1312-1986. Displayed by screenshot of the Nextstrain software.

The purple colour indicates movement from South Africa and the light brown colour indicate movement from Zimbabwe. The animated movement can be found at *http://bioinformatic.solutions:9999/.* With permission from under Open Street Map license: https://www.openstreetmap.org/copyright

Figure.4 Directional movement of DR-TB L2 strain showing amino acid changes, 1989, Zimbabwe and South Africa. Displayed by screenshot of the Nextstrain software. The animated movement can be found at *http://bioinformatic.solutions:9999/.* The purple indicates movement from South African provinces to Zimbabwe and the brown indicate movement from Zimbabwe to South Africa. With permission from under Open Street Map license: https://www.openstreetmap.org/copyright

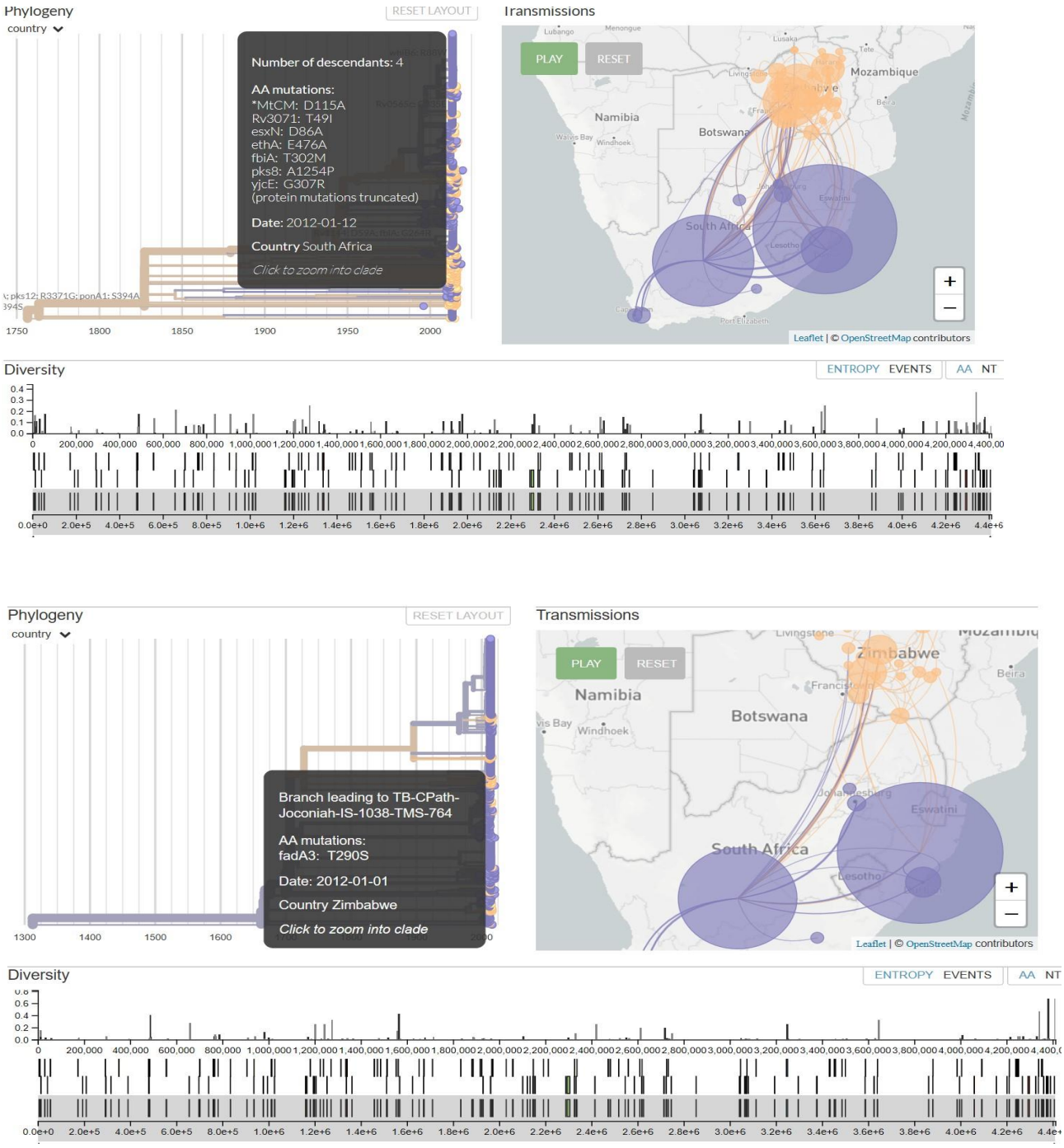


Figure.5 Directional movement of DR-TB L2 strains showing amino acid changes for period 2011-2012, Zimbabwe and South Africa. Displayed by screenshot of the Nextstrain software. The animated movement can be found at http://bioinformatic.solutions:9999/. The purple indicates movement from South African provinces to Zimbabwe and the brown indicate movement from Zimbabwe to South Africa. With permission from under Open Street Map license: https://www.openstreetmap.org/copyright

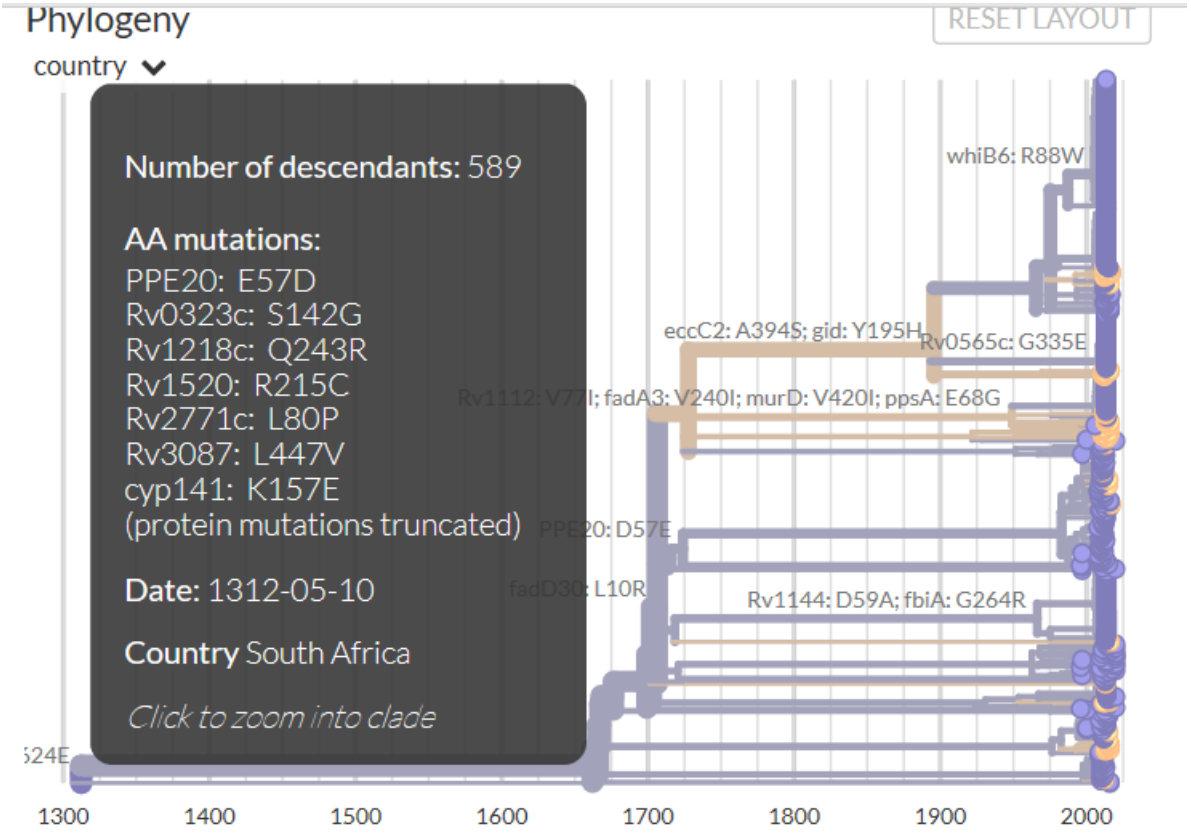


Figure.6 Phylogeography tree of DR-TB L2 strains showing amino acid changes in 1312. Displayed by screenshot of the Nextstrain software. The animated movement can be found at at http://bioinformatic.solutions:9999/.

References

1. WHO WHO. Global Tuberculosis Report. Geneva, Switzerland; 2018.

2. World Health Organization. Global tuberculosis report 2017. 2017.

3. Billo NE. Costs for TB and MDR-TB: time to convene a WHO Emergency Committee on TB? Int J Tuberc Lung Dis. 2016 Apr 1;20(4):427–427.

4. Brummer D. Labour migration and HIV/AIDS in southern Africa. Int Organ Migr Reg Off South Afr [Internet]. 2002 [cited 2017 Jan 20];

5. Dodson B, Crush J. Migration Governance and Migrant Rights in the Southern African Development Community (SADC). :27.

6. Matteelli A, Lönnroth K, Mosca D, Getahun H, Centis R, D'Ambrosio L, et al. Cameroon's multidrug-resistant tuberculosis treatment programme jeopardised by cross-border migration. Eur Respir J. 2016 Feb;47(2):686–8.

7. Barroso EC, Mota RMS, Santos RO, Sousa ALO, Barroso JB, Rodrigues JLN. Risk factors for acquired multidrug-resistant tuberculosis. J Pneumol. 2003;29(2):89–97.

8. Atre S, D'Souza DesireeTB, Vira T, Chatterjee A, Mistry N. Risk factors associated with MDR-TB at the onset of therapy among new cases registered with the RNTCP in Mumbai, India. Indian J Public Health. 2011;55(1):14.

9. Filliol I, Motiwala AS, Cavatore M, Qi W, Hazbon MH, Bobadilla del Valle M, et al. Global Phylogeny of Mycobacterium tuberculosis Based on Single Nucleotide Polymorphism (SNP) Analysis: Insights into Tuberculosis Evolution, Phylogenetic Accuracy of Other DNA Fingerprinting Systems, and Recommendations for a Minimal Standard SNP Set. J Bacteriol. 2006 Jan 15;188(2):759–72.

10. Gagneux S, Small PM. Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. Lancet Infect Dis. 2007 May;7(5):328–37.

11. Cohen KA, Manson AL, Abeel T, Desjardins CA, Chapman SB, Hoffner S, et al. Extensive global movement of multidrug-resistant *M. tuberculosis* strains revealed by whole-genome analysis. Thorax. 2019 Sep;74(9):882–9.

12. Cooke G, Beaton RK, Lessells R, John L, Ashworth S, Kon O, et al. International Spread of MDR TB from Tugela Ferry, South Africa. Emerg Infect Dis [Internet]. 2011 Nov [cited 2017 Feb 7];17(11).

13. Hargreaves S, Lönnroth K, Nellums LB, Olaru ID, Nathavitharana RR, Norredam M, et al. Multidrug-resistant tuberculosis and migration to Europe. Clin Microbiol Infect. 2017 Mar;23(3):141–6.

14. Noeske, J, Abena J-L, Kuaban. Cameroon MDR-TB treatment programme can be jeopardised by cross-border migration.

15. Cain KP, Marano N, Kamene M, Sitienei J, Mukherjee S, Galev A, et al. The movement of multidrug-resistant tuberculosis across borders in East Africa needs a regional and global solution. PLoS Med. 2015;12(2):e1001791.

16. Gehre F, Kumar S, Kendall L, Ejo M, Secka O, Ofori-Anyinam B, et al. A Mycobacterial Perspective on Tuberculosis in West Africa: Significant Geographical Variation of M. africanum and Other M. tuberculosis Complex Lineages. PLoS Negl Trop Dis. 2016;10(3):e0004408.

17. Lurie MN, Williams BG. Migration and health in Southern Africa: 100 years and still circulating. Health Psychol Behav Med Open Access J. 2014;2(1):34–40.

18. Crush J, Williams V, Peberdy S. Migration in southern Africa. Policy Anal Res Programme Glob Comm Int Migr [Internet]. 2005 [cited 2017 Feb 7];

19. Fitchett JR, Vallecillo AJ, Espitia C. Tuberculosis transmission across the United States-Mexico border. Rev Panam Salud Pública. 2011 Jan;29(1):57–60.

20. Golesi F, Brignatz J, Bellenfant M, Raoult D, Drancourt M. Mycobacterium tuberculosis Beijing outbreak in a school in Marseille, France, 2012. Euro Surveill [Internet]. 2013 [cited 2015 Apr 10];18(2).

21. Marais BJ, Mlambo CK, Rastogi N, Zozio T, Duse AG, Victor TC, et al. Epidemic Spread of Multidrug-Resistant Tuberculosis in Johannesburg, South Africa. J Clin Microbiol. 2013 Jun 1;51(6):1818–25.

22. Crush J, Tawodzera G. The Food Insecurities of Zimbabwean Migrants in Urban South Africa [Internet]. Southern African Migration Programme; 2016 [cited 2019 Jul 26].

23. Said HM, Kock MM, Ismail NA, Mphahlele M, Baba K, Omar SV, et al. Molecular Characterization and Second-Line Antituberculosis Drug Resistance Patterns of Multidrug-Resistant Mycobacterium tuberculosis Isolates from the Northern Region of South Africa. J Clin Microbiol. 2012 Sep 1;50(9):2857–62.

24. Mogashoa T, Melamu P, Ley SD, Streicher EM, Iketleng T, Kelentse N, et al. Genetic diversity of Mycobacterium tuberculosis strains circulating in Botswana. Hozbor DF, editor. PLOS ONE. 2019 May 7;14(5):e0216306.

25. Johnson R, Warren R, Strauss OJ, Jordaan AM, Beyers N, Schaaf HS, et al. An outbreak of drug-resistant tuberculosis caused by a Beijing strain in the Western Cape, South Africa [Short Communication]. Int J Tuberc Lung Dis. 2006;10(12):1412–1414.

26. Takarinda KC, Sandy C, Masuka N, Hazangwe P, Choto RC, Mutasa-Apollo T, et al. Factors Associated with Mortality among Patients on TB Treatment in the Southern Region of Zimbabwe, 2013. Tuberc Res Treat. 2017;2017.

27. Timire C, Sandy C, Kumar AMV, Ngwenya M, Murwira B, Takarinda KC, et al. Access to second-line drug susceptibility testing results among patients with Rifampicin resistant tuberculosis after introduction of the Hain Line Probe Assay in Southern provinces, Zimbabwe. Int J Infect Dis. 2019 Apr;81:236–43.

28. Timire C, Takarinda KC, Harries AD, Mutunzi H, Manyame-Murwira B, Kumar AMV, et al. How has the Zimbabwe mycobacterial culture and drug sensitivity testing system among re-treatment tuberculosis patients functioned during the scale-up of the Xpert MTB/RIF assay? Trans R Soc Trop Med Hyg. 2018 Jun 1;112(6):285–93.

29. Dowdy DW, Chaisson RE, Maartens G, Corbett EL, Dorman SE. Impact of enhanced tuberculosis diagnosis in South Africa: A mathematical model of expanded culture and drug susceptibility testing. Proc Natl Acad Sci. 2008 Aug 12;105(32):11293–8.

30. Department of Health, Republic of South Africa. Iinterim Clinica; guidelines for the implementation of injectable free regimens for rifampicin resistant tuberculosis in adults adolescents and children. Government of South Arica; 2018.

31. National Insitute of Health D of H and HS. National Center for Biotechnology Information, National Library of Medicine, SRA. Fact sheet.pdf. National Institutes of Health • Department of Health and Human Services; 2015.

32. Griffing SM, MacCannell DR, Schmidtke AJ, Freeman MM, Hyytiä-Trees E, Gerner-Smidt P, et al. Canonical Single Nucleotide Polymorphisms (SNPs) for High-Resolution Subtyping of Shiga-Toxin Producing Escherichia coli (STEC) O157:H7. Dobrindt U, editor. PLOS ONE. 2015 Jul 1;10(7):e0131967.

33. Comas I, Hailu E, Kiros T, Bekele S, Mekonnen W, Gumi B, et al. Population genomics of Mycobacterium tuberculosis in Ethiopia contradicts the virgin soil hypothesis for human tuberculosis in Sub-Saharan Africa. Curr Biol. 2015;25(24):3260–3266.

34. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15;25(14):1754–60.

35. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep 1;20(9):1297–303.

36. Delcher AL. Fast algorithms for large-scale genome alignment and comparison. Nucleic Acids Res. 2002 Jun 1;30(11):2478–83.

37. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016 Jul;33(7):1870–4.

38. Colman RE, Anderson J, Lemmer D, Lehmkuhl E, Georghiou SB, Heaton H, et al. Rapid Drug Susceptibility Testing of Drug-Resistant Mycobacterium tuberculosis Isolates Directly from Clinical Samples by Use of Amplicon Sequencing: a Proof-of-Concept Study. Land GA, editor. J Clin Microbiol. 2016 Aug;54(8):2058–67.

39. Bowers JR, Lemmer D, Sahl JW, Pearson T, Driebe EM, Wojack B, et al. KlebSeq, a Diagnostic Tool for Surveillance, Detection, and Monitoring of Klebsiella pneumoniae. Ledeboer NA, editor. J Clin Microbiol. 2016 Oct;54(10):2582–96.

40. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol. 2015 Jan;32(1):268–74.

41. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Kelso J, editor. Bioinformatics. 2018 Dec 1;34(23):4121–3.

42. Kumar S, Stecher G, Suleski M, Hedges SB. TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. Mol Biol Evol. 2017 Jul;34(7):1812–9.

43. Chihota V, Apers L, Mungofa S, Kasongo W, Nyoni IM, Tembwe R, et al. Predominance of a single genotype of Mycobacterium tuberculosis in regions of Southern Africa. Int J Tuberc Lung Dis. 2007;11(3):311–318.

44. Eldholm V, Monteserin J, Rieux A, Lopez B, Sobkowiak B, Ritacco V, et al. Four decades of transmission of a multidrug-resistant Mycobacterium tuberculosis outbreak strain. Nat Commun. 2015 May 11;6:7119.

45. Cheng S, Chen W, Yang Y, Chu P, Liu X, Zhao M, et al. Effect of Diagnostic and Treatment Delay on the Risk of Tuberculosis Transmission in Shenzhen, China: An Observational Cohort Study, 1993–2010. Dowdy DW, editor. PLoS ONE. 2013 Jun 27;8(6):e67516.

46. Rodríguez-Castillo JG, Pino C, Niño LF, Rozo JC, Llerena-Polo C, Parra-López CA, et al. Comparative genomic analysis of Mycobacterium tuberculosis Beijing-like strains revealed specific genetic variations associated with virulence and drug resistance. Infect Genet Evol. 2017 Oct;54:314–23.

47. Grandjean L, Gilman RH, Martin L, Soto E, Castro B, Lopez S, et al. Transmission of Multidrug-Resistant and Drug-Susceptible Tuberculosis within Households: A Prospective Cohort Study. Cobelens F, editor. PLOS Med. 2015 Jun 23;12(6):e1001843.

48. Murray J1, Sonnenberg P, Godfrey-Faussett P, Shearer S,. Drug-resistant pulmonary tuberculosis in a cohort of southern African goldminers with a high prevalence of HIV infection. Afr Med J. 2000 Apr;90(4):381–6.

49. Merker M, Blin C, Mona S, Duforet-Frebourg N, Lecher S, Willery E, et al. Evolutionary history and global spread of the Mycobacterium tuberculosis Beijing lineage. Nat Genet. 2015 Mar;47(3):242–9.

50. Guglielmetti L, Varaine F, Huerga H, Bonnet M, Rich ML, Mitnick CD. Shortened multidrug-resistant tuberculosis treatment in settings with a high prevalence of ofloxacin resistance. Eur Respir J. 2017 Jul;50(1):1700598.

51. Bloemberg GV, Keller PM, Stucki D, Trauner A, Borrell S, Latshang T, et al. Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. N Engl J Med. 2015 Nov 12;373(20):1986–8.

52. Nguyen TVA, Anthony RM, Bañuls A-L, Nguyen TVA, Vu DH, Alffenaar J-WC. Bedaquiline Resistance: Its Emergence, Mechanism, and Prevention. Clin Infect Dis. 2018 May 2;66(10):1625–30.

53. de Vos M, Ley SD, Wiggins KB, Derendinger B, Dippenaar A, Grobbelaar M, et al. Bedaquiline Microheteroresistance after Cessation of Tuberculosis Treatment. N Engl J Med. 2019 May 30;380(22):2178–80.

54. Field SK. Bedaquiline for the treatment of multidrug-resistant tuberculosis: great promise or disappointment? Ther Adv Chronic Dis. 2015 Jul;6(4):170–84.

55. Andries K, Villellas C, Coeck N, Thys K, Gevers T, Vranckx L, et al. Acquired Resistance of Mycobacterium tuberculosis to Bedaquiline. van Veen HW, editor. PLoS ONE. 2014 Jul 10;9(7):e102135.

56. Veziris N, Bernard C, Guglielmetti L, Le Du D, Marigot-Outtandy D, Jaspard M, et al. Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons to avoid repeating past errors. Eur Respir J. 2017 Mar;49(3):1601719.

57. Tiwari BM, Kannan N, Vemu L, Raghunand TR. The Mycobacterium tuberculosis PE Proteins Rv0285 and Rv1386 Modulate Innate Immunity and Mediate Bacillary Survival in Macrophages. Briken V, editor. PLoS ONE. 2012 Dec 17;7(12):e51686.

58. Mphahlele M, Syre H, Valvatne H, Stavrum R, Mannsaker T, Muthivhi T, et al. Pyrazinamide Resistance among South African Multidrug-Resistant Mycobacterium tuberculosis Isolates. J Clin Microbiol. 2008 Oct 1;46(10):3459–64.

59. Zhang Y, Shi W, Zhang W, Mitchison D. Mechanisms of Pyrazinamide Action and Resistance. Microbiol Spectr [Internet]. 2014 Aug 22 [cited 2019 Oct 28];2(4).

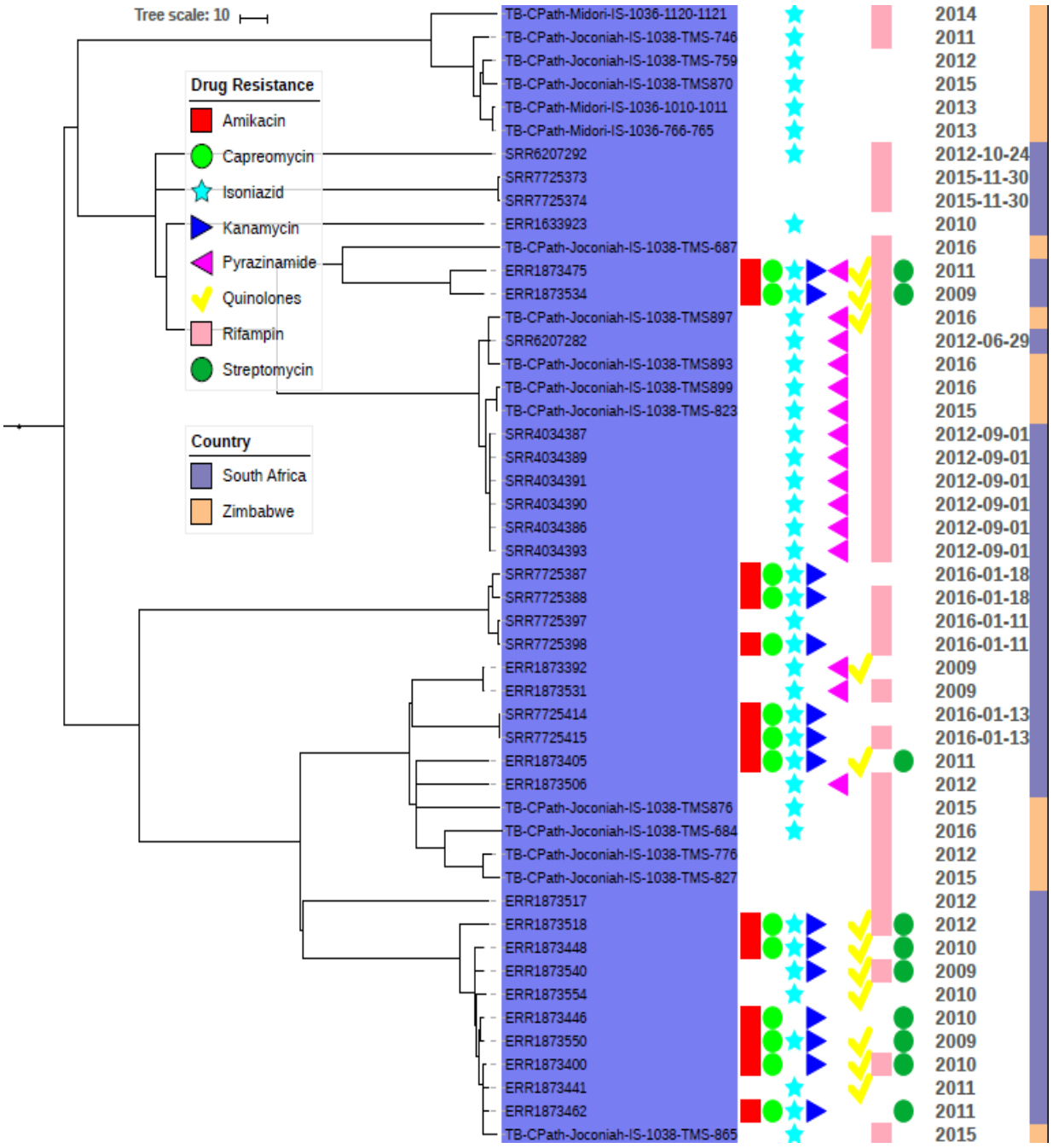
60. Xiao H. Pyrazinamide resistance in MDR and extensively drug-resistant tuberculosis. Biomed Res. 2017;28(8):5.

61. Scheld WM. Maintaining Fluoroquinolone Class Efficacy: Review of Influencing Factors. Emerg Infect Dis. 2003 Jan;9(1):1–9.

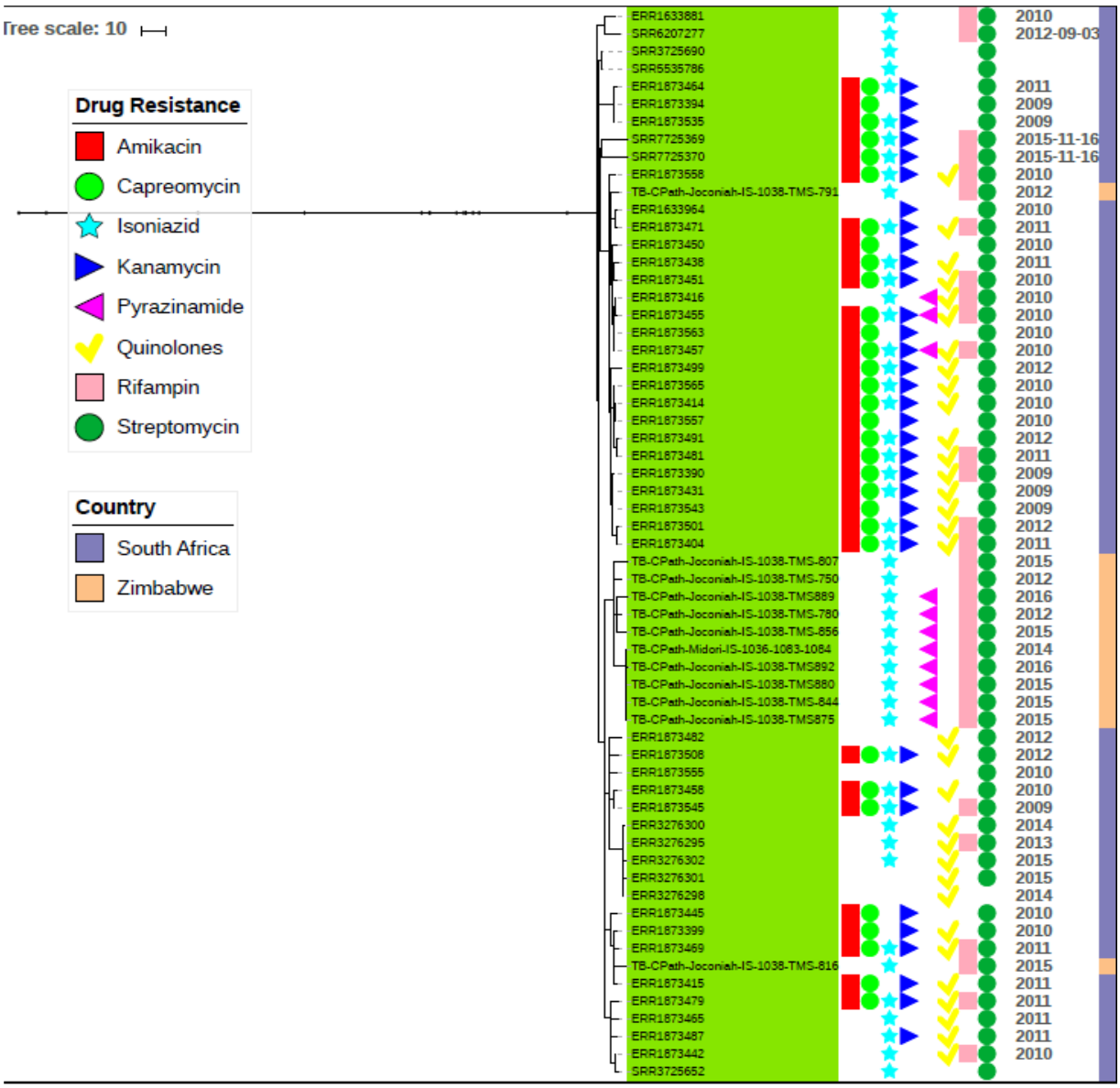
62. Mashe T, Gudza-Mugabe M, Tarupiwa A, Munemo E, Mtapuri-Zinyowera S, Smouse SL, et al. Laboratory characterisation of Salmonella enterica serotype Typhi isolates from Zimbabwe, 2009–2017. BMC Infect Dis. 2019 Dec;19(1):487.

63. Bernard C, Brossier F, Sougakoff W, Veziris N, Frechet-Jachym M, Metivier N, et al. A surge of MDR and XDR tuberculosis in France among patients born in the Former Soviet Union. Eurosurveillance [Internet]. 2013 Aug 15 [cited 2019 Feb 14];18(33).

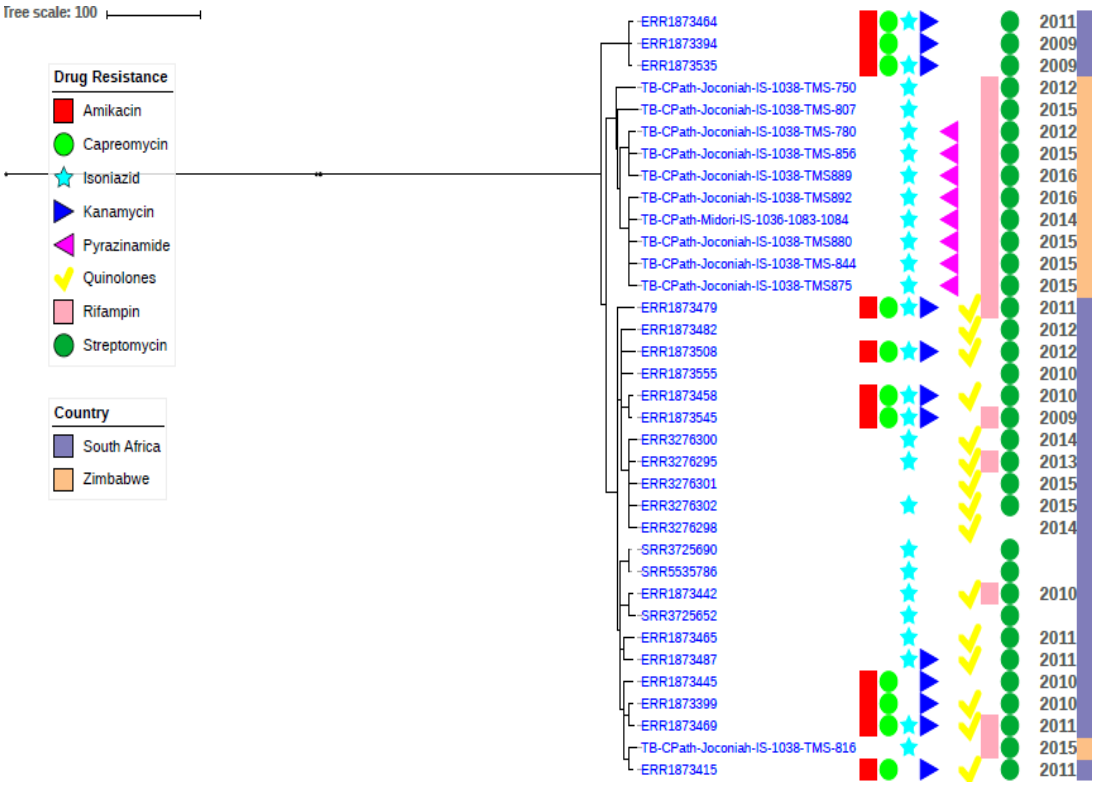
Supplemental figures



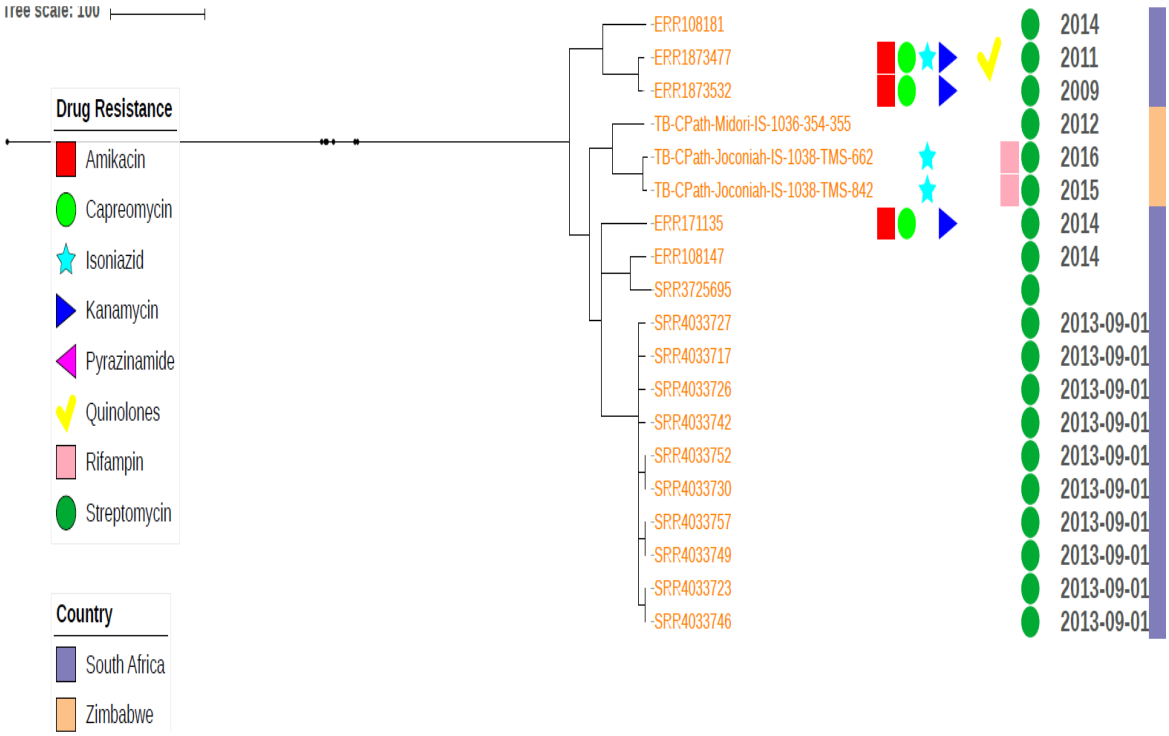
Supplemental Figure 1a: Extract of DR-TB lineage 4 (L4) strains at 10 o'clock position of figure 1a showing between country relatedness and resistance patterns, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life (ITOL) software, https://itol.embl.de/shared/corburn



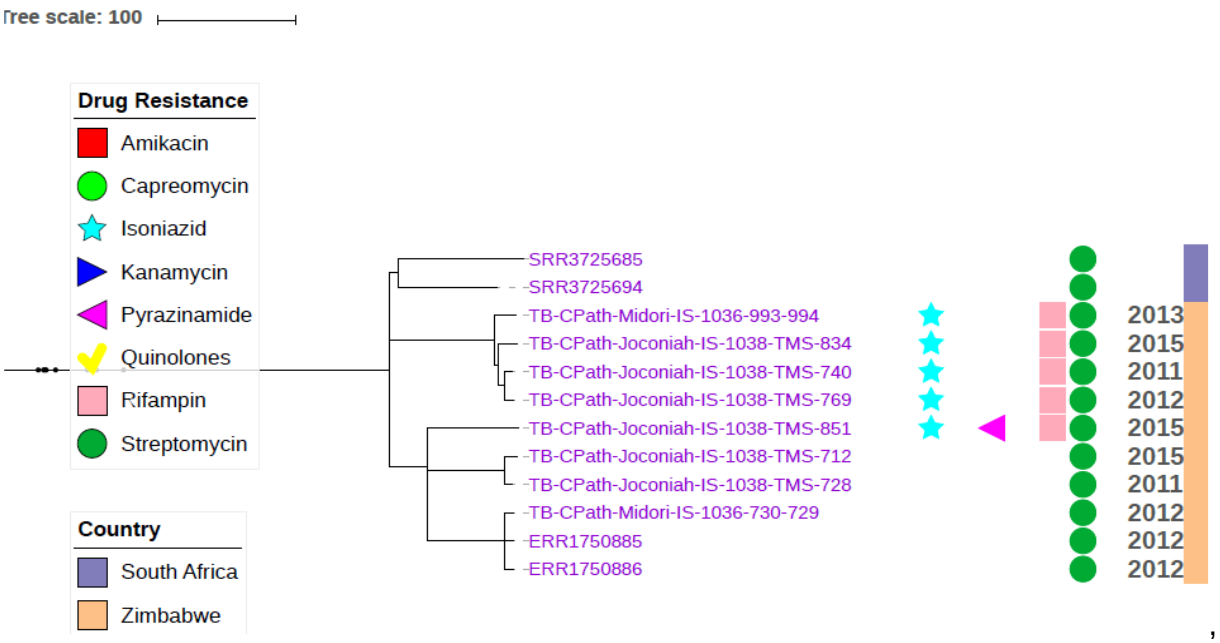
Supplemental Figure 1b. Extract of DR-TB L2 strains at 4 o'clock position of figure 1a showing between country relatedness and resistance pattern, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life (ITOL) software, https://itol.embl.de/shared/corburn



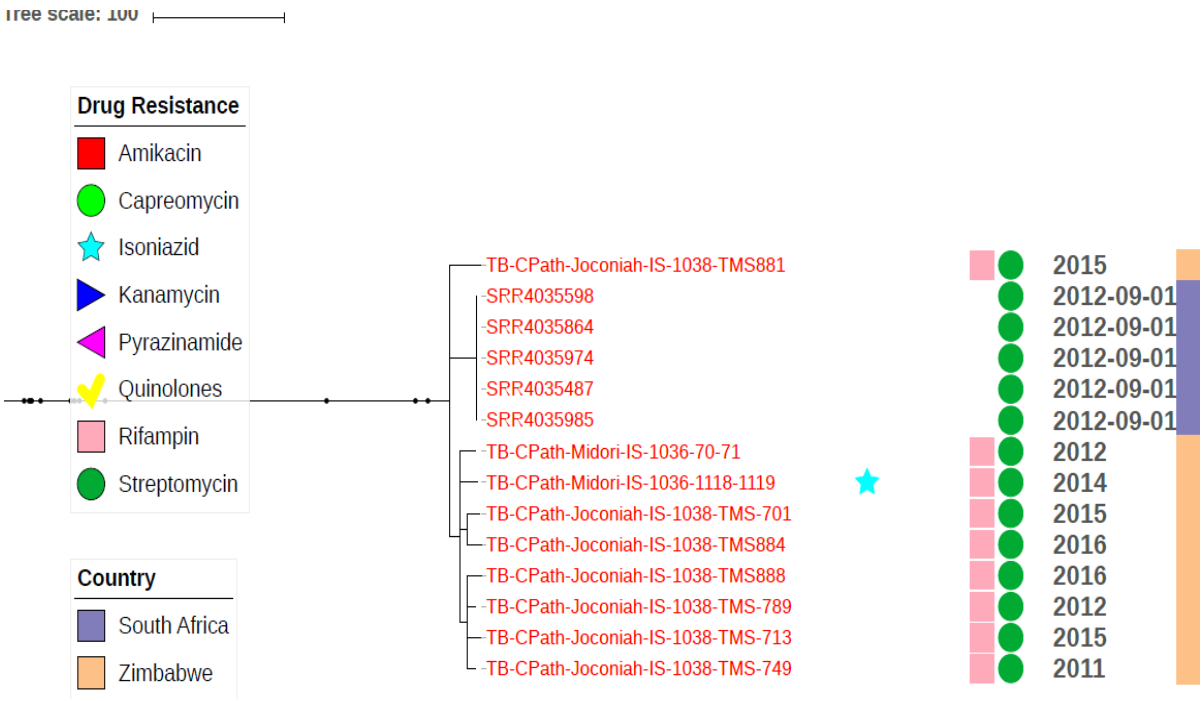
Supplemental Figure 2a. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn



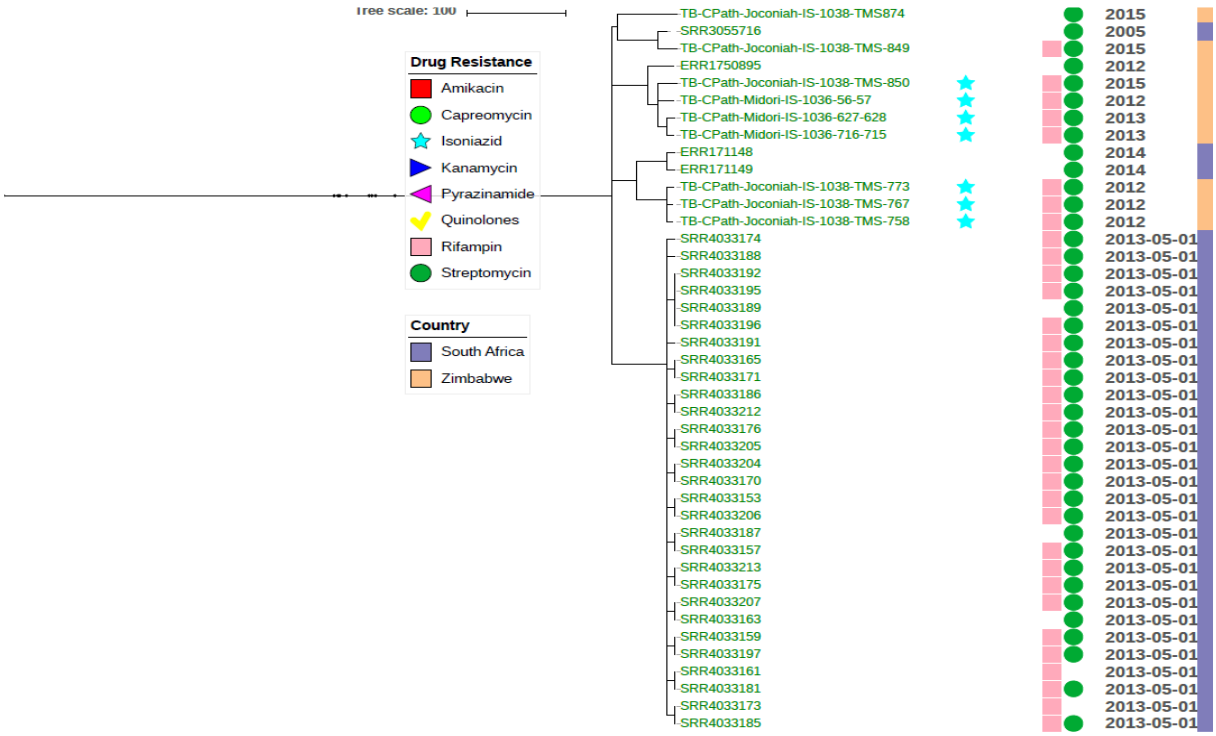
Supplemental Figure 2b. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn



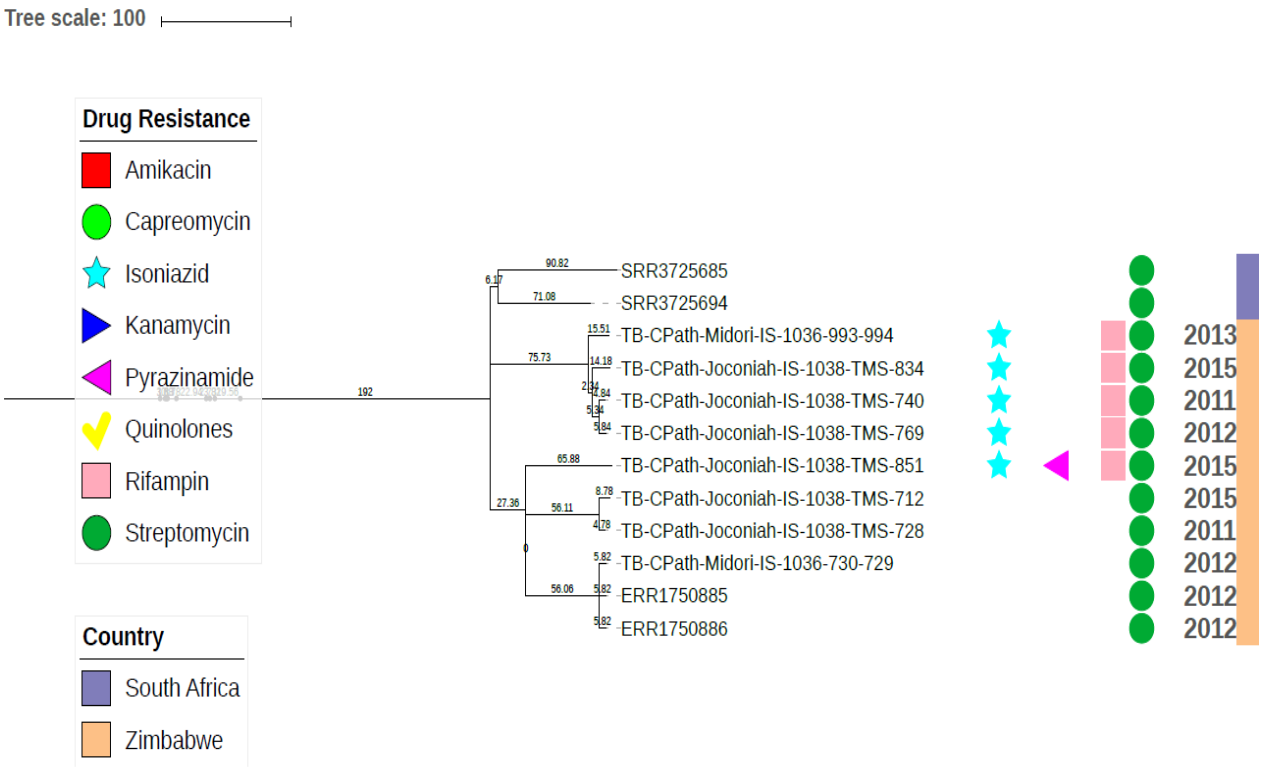
Supplemental Figure 2c. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn



Supplemental Figure 2d. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn



Supplemental Figure 2e. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn



Supplemental Figure 2f. Phylogenetic tree showing the temporal clustering of L2 strains as a subset of figure 2a, by resistance profile, time of diagnosis, branch lengths and country, Zimbabwe and South Africa. Displayed by screenshot of Interactive Tree of Life software, https://itol.embl.de/shared/corburn

Chapter 8

General conclusions and recommendations

This chapter summarises the general findings, interpretation and overall recommendations of the thesis

8.0 Summary of Findings and Conclusions

8.1 Introduction

Globalization of infectious diseases from population movement, is an established phenomenon (1,2). Studies that have described the epidemiology of tuberculosis (TB) spread through population movement have been limited to high income countries (3). High income countries have adequate research capacity and strong public health infrastructure to support implementation of migration and infectious diseases studies. As a result, the key recommendations to control and prevent the spread of infectious diseases arising from migration remain relevant to high income countries but may not translate to resource limited settings (4). Capacity to screen, diagnose and treat infectious immigrants is often inadequate in most low and middle income countries (LMIC) (1,5).

Cross-border movement of populations in the Southern Africa Development Community (SADC) region is known to be high (6). The current epidemiology of both human immunodeficiency virus (HIV) and TB infection is believed to have been shaped by the historical labour migration in the region (7). Studies of TB among mine workers in South Africa confirmed spread of TB between mine and non-mine workers (10). There were knowledge gaps on the magnitude of the burden of TB transmission due to migration between high burden countries. With the increasing prevalence of rifampicin resistant/multidrug resistant TB (RR/MDR-TB) in the SADC region, understanding the transmission dynamics of migration-related TB became an urgent public health need (9). To our knowledge this is the first in-depth study on cross-border spread of TB in the SADC region.

8.2 Key conclusions and recommendations

Published literature on RR/MDR-TB transmission and migration remain skewed towards high income where capacity for second line drug sensitivity testing (SLDST) is available. Even in the high income countries evidence in actual transmission between immigrants and natives was minimal, mainly because of low mixing patterns of populations with different cultural backgrounds (3). Two observations may inform decision to invest in both health systems strengthening and social determinants of health in low income countries. First was the observation that immigrants' risk to RR/MDR-TB infection and disease significantly reduced after five years of migrating to high income countries. This means investment in social determinants of health like social protection and physical planning in low income have impact on the burden of TB (10,11). The second observation was the favourable treatment outcomes that immigrants with RR/MDR-TB had after treatment (12,13). Health systems in high income countries have capacity to ensure early diagnosis and treatment of RR/MDR-TB patients which promotes favourable treatment outcome (14). Investing in diagnostic capacity in low income countries like Zimbabwe would facilitate early diagnosis and treatment for RR/MDR-TB patients and improve the low treatment success rate of 51%(15).

This study confirmed that TB outbreaks do occur and have similar characteristics as acute infectious diseases with propagated transmission if no control measures are put in place. Consideration of the high clustering and hotspot phenomenon demonstrated in poor peri-urban settlements in Harare city are important for designing health care services and programmes to control TB transmission in similar settings. The Zimbabwe public health system is based on provision of services using the primary health care system approach, which prioritise preventive services primarily in rural

areas where 70% of the population lives (16). Our findings confirm that peri-urban poor populations are at-risk populations to poverty related diseases like TB and must be prioritised in terms of health service delivery, in the same way as rural populations in Zimbabwe. Harare City council is encouraged to engage with the Ministry of Health and Child Care (MoHCC) in Zimbabwe to mobilize resources for the construction of a health centre in South West District to improve access to health care services.

One of the reasons for limited research capacity in high TB burden settings like Zimbabwe has been limitations of the laboratory to support clinical research (17). As technology improves, the use of historical biosamples to study either occurrence of known diseases or genetic changes of known pathogens is a standard practice (18). Current guidelines for the storage of sputum samples for future molecular epidemiological research studies are based on guidelines developed in 1972 (19,20). Our study provided some evidence that sputum isolates stored at room temperature in MGIT tubes for up to 6 years could remain useful for future molecular studies. A similar study to ours from China demonstrated that clinical Mtb isolates could be stored in MGIT tubes for a maximum of two years but less than six years (21). In resource-limited environments where there is erratic supply of electricity, and therefore limited capacity to store samples at sub-zero levels and limited sputum culture facilities, our findings will agitate for the revision of current guidelines and allow low income countries to store and use TB samples using cheaper methods. However, the need for a proper TB sample biobanking system was an urgent recommendation to improve TB infection prevention and control in the laboratory and improve the viability of *Mycobacterium tuberculosis* in the stored sputum. A study under experimental conditions to determine the optimum conditions for sputum sample storage at room temperature is recommended.

Thirdly, this study confirmed that there was active RR/MDR-TB transmission during and after cross border movement of people involving two high burden countries. This was demonstrated by the increasing population of Beijing strains in Zimbabwe, where historically, mainly LAM11_ZWE had been the predominant genotype (22). Whilst the increasing population of the T and S genotype in the Southern region of the country could be inferred to migration between Zimbabwe and Botswana where similar genotypes were also common, we could not confirm if these also had similar characteristics of Beijing to occur in epidemic form. The combination of the Beijing, T, S and LAM11_ZWE genotypes in Zimbabwe among RR/MDR-TB patients provided a baseline for future studies to track RR/MDR-TB transmission in the country. Predominance of the Beijing, T and S in the southern region more than the northern Zimbabwe could be explained by the strong cultural and ancestral linkages of the southern populations with South Africa (23). There may be however need for determining presence of genetic predisposition of the Southern population to Beijing strain infection in addition to the higher migration patterns (24). A coordinated approach in cross-border prevention and control of RR/MDR-TB is urgently required in the SADC region to contain the epidemic. Current efforts targeting mine workers and ex-mine workers may not be adequate given the nature of migration and high mixing patterns. The available draft guidelines on the management of TB, Malaria and HIV in the SADC region should be revitalised and operationalised with urgency to include improving access to health care services irrespective of immigrant status (14).

Zimbabwe started reporting extremely DR-TB (XDR-TB) in 2017 (26). Whole genome sequencing results confirmed that about 5% of the RR/MDR-TB cases were pre-XDR-TB. With the inadequate laboratory capacity where the country was not able to provide second line drug sensitivity testing (DST), treatment of RR/MDR-TB patients was

being commenced based on Gene Xpert results and only identified as failure after several months of treatment follow up. The suboptimal second line drug regimens may be potentiating development of XDR-TB in Zimbabwe (27). Building laboratory capacity for second line DST in Zimbabwe is an urgent requirement, especially as the country plans to transition to the new shorter treatment regimen according to the recent WHO recommendation (28). We strongly recommended that Zimbabwe introduces new TB diagnostic techniques that will facilitate anti-TB drug sensitivity analysis to allow the use of the most efficacious drug regimen.

Lastly, a Zimbabwe-South Africa phylogeographic study showed that the Beijing genotype was introduced in South Africa centuries ago and has subsequently spread to Zimbabwe. Presence of mutations that could confer resistance to the new anti TB drugs, BDQ, Delamanid and Clofazimine were observed in Zimbabwean strains. Development of DR TB may therefore not be related to programmatic causes alone as currently believed (29,30). Continuous research on mechanism of anti-TB drug resistance development is therefore required. In addition, there is an urgent need to continue working on new anti-TB drug development.

8.3 Lessons from the study

We highlight a few lessons learnt from implementation of this study. A major lesson was the inadequate policy mechanism to facilitate collaborative cross border research. Obtaining regulatory approvals for specimen transmittal agreements from Zimbabwe to South Africa was administratively arduous and time consuming. While ultimately this had minimal impact on the quality of the laboratory work and overall output of the study, these challenges have highlighted lessons for future research. Firstly, to allow regional research collaboration for combating migration-related MDR-TB, a minimum level of laboratory capacity is required for all partner institutions. This will allow the

basic processing of the samples like DNA extraction and spoligotyping. Specimen transmittal of the DNA has less restrictive legal requirements than the whole specimen. Secondly, Stellenbosch University legal unit has qualified people dealing with inter-institutional agreements. In Zimbabwe a separate government department mandated to regulate collaborations with external institutions did not have dedicated legal personnel to deal with emerging issues in research regulations. This affected the timeous resolution of issues. Through the responsible departmental ministry, there is urgent need to improve the research governance system at the University of Zimbabwe. Research regulatory bodies should improve on responsiveness to minimize challenges related with regional and international research collaboration in line with their mandate of promoting innovation through research whilst protecting the local researchers.

After decentralization of the clinical management of RR/MDR-TB, one of the national reference laboratories from the northern region was upgraded to become a national TB reference laboratory. The quality of samples from this laboratory was inadequate compared to the southern region designated National TB reference laboratory (NTRL). As a result, more samples from the southern region were recovered. In addition, retrieval of samples from the northern laboratory was limited. This may have affected the representativeness of the RR/MDR-TB isolates available for analysis. We strongly recommend that the Zimbabwe MoHCC must strengthen the TB laboratory capacity especially as the country plans to roll out the new shorter treatment regimen.

Several research questions arising from our findings require a coordinated process to lead the development of a TB research consortium in Zimbabwe. The medical schools in Zimbabwe, should take leadership in TB research activities and provide answers to the emerging issues on RR/MDR-TB in the country. Collaboration with regional

academic institutions could assist in initial capacity development in terms of human and infrastructure. The research group must be responsible for resource mobilization in addition to the scientific leadership.

Based on these findings, we propose that as a minimum, Zimbabwe and South Africa collaborate on the following future but urgent studies. First, a study on BDQ and Delamanid resistance is probably urgent given the anecdotal evidence from Zimbabwe and research findings from South Africa. Second, the extent and effect of pyrazinamide resistance on the treatment of susceptible TB in Zimbabwe will need more research. Third, the feasibility of joint surveillance programme for DR-TB between South Africa and Zimbabwe using whole genome sequencing, along the European Union model could benefit the two countries to understand the extend of the DR-TB epidemic and develop appropriate treatment regimens (31).

References

1. Gushulak BD, MacPherson DW. Globalization of Infectious Diseases: The Impact of Migration. Clin Infect Dis. 2004 Jun 15;38(12):1742–8.

2. Lance S, 3. KL, 4. BK, 5. AG, 6., 7., et al. Globalization and infectious diseases: A review of the linkages. World Health Organization; 2004.

3. Pareek M, Greenaway C, Noori T, Munoz J, Zenner D. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. BMC Med [Internet]. 2016 Dec [cited 2017 Jan 29];14(1).

4. Soto SM. Human migration and infectious diseases. Clin Microbiol Infect. 2009 Jan;15:26–8.

5. Castelli F, Sulis G. Migration and infectious diseases. Clin Microbiol Infect. 2017 May;23(5):283–9.

6. Crush J, Williams V, Peberdy S. Migration in southern Africa. Policy Anal Res Programme Glob Comm Int Migr [Internet]. 2005 [cited 2017 Feb 7];

7. Brummer D. Labour migration and HIV/AIDS in southern Africa. Int Organ Migr Reg Off South Afr [Internet]. 2002 [cited 2017 Jan 20];

8. Mathema B, Lewis JJ, Connors J, Chihota VN, Shashkina E, van der Meulen M, et al. Molecular Epidemiology of *Mycobacterium tuberculosis* among South African Gold Miners. Ann Am Thorac Soc. 2015 Jan;12(1):12–20.

9. Gandhi NR, Brust JCM, Moodley P, Weissman D, Heo M, Ning Y, et al. Minimal Diversity of Drug-Resistant *Mycobacterium tuberculosis* Strains, South Africa1. Emerg Infect Dis. 2014 Mar;20(3):394–401.

10. Hargreaves JR, Boccia D, Evans CA, Adato M, Petticrew M, Porter JDH. The Social Determinants of Tuberculosis: From Evidence to Action. Am J Public Health. 2011 Apr;101(4):654–62.

11. Heiman HJ, Artiga S. Beyond health care: the role of social determinants in promoting health and health equity. Health (N Y) [Internet]. 2016 [cited 2017 Mar 5];20(10).

12. Ward HA, Marciniuk DD, Hoeppner VH, Jones W. Treatment outcome of multidrug-resistant tuberculosis among Vietnamese immigrants. Int J Tuberc Lung Dis. 2005;9(2):164–169.

13. Mor Z, Goldblatt D, Kaidar-Shwartz H, Cedar N, Rorman E, Chemtob D. Drug-resistant tuberculosis in Israel: risk factors and treatment outcomes. Int J Tuberc Lung Dis. 2014 Oct 1;18(10):1195–201.

14. Harris RC, Grandjean L, Martin LJ, Miller AJP, Nkang J-EN, Allen V, et al. The effect of early versus late treatment initiation after diagnosis on the outcomes of patients treated for multidrug-resistant tuberculosis: a systematic review. BMC Infect Dis [Internet]. 2016 Dec [cited 2017 Feb 2];16(1).

15. World Health Organization. Global tuberculosis report 2017. 2017.

16. Ministry of Health and child care. Zimbabwe National Health Strategy: Equity and quality in health; Leaving noone behind, 2016-2020. Government of Zimbabwe; 2016.

17. Alabi AS, Traoré AN, Loembe MM, Ateba-Ngoa U, Frank M, Adegnika AA, et al. Enhanced laboratory capacity development: a boost for effective tuberculosis control in resource-limited settings. Int J Infect Dis. 2017 Mar;56:81–4.

18. Donoghue HD. Insights gained from palaeomicrobiology into ancient and modern tuberculosis. Clin Microbiol Infect. 2011 Jun;17(6):821–9.

19. KUBICA, GP, GONTIJO-FILHO PP, KIM T. Preservation of Mycobacteria at -70°C: Persistence of Key Differential Features. J Clin Microbiol. 1977 Aug;Vol. 6,(No. 2):149–53.

20. KIM TH, KUBICA, GP. Long-Term Preservation and Storage of Mycobacteria. Appl Microbiol. 1972;Vol. 24,(No. 3):311–7.

21. Huang T-S, Chen Y-S, Lee SS-J, Tu H-Z, Liu Y-C. Preservation of Clinical Isolates of Mycobacterium tuberculosis Complex Directly from MGIT Culture Tubes. 2005;35(4,).

22. Chihota V, Apers L, Mungofa S, Kasongo W, Nyoni IM, Tembwe R, et al. Predominance of a single genotype of Mycobacterium tuberculosis in regions of Southern Africa. Int J Tuberc Lung Dis. 2007;11(3):311–318.

23. Mlambo AS. History of Zimbabwe Migration.pdf [Internet]. Univerity of Pretoria; 2010 [cited 2018 Nov 30].

24. van Crevel R, Parwati I, Sahiratmadja E, Marzuki S, Ottenhoff THM, Netea MG, et al. Infection with *Mycobacterium tuberculosis* Beijing Genotype Strains Is Associated with Polymorphisms in *SLC11A1/NRAMP1* in Indonesian Patients with Tuberculosis. J Infect Dis. 2009 Dec;200(11):1671–4.

25. Southern African Development Community, Directorate of Social HD& SP. Harmonised minimum standards for the prevention, treatment and management of tuberculosis in the SADC region. 2010.

26. World Health Organization. Global tuberculosis report 2018. 2018.

27. Seddon JA, Warren RM, Enarson DA, Beyers N, Schaaf HS. Drug-Resistant Tuberculosis Transmission and Resistance Amplification within Families. Emerg Infect Dis [Internet]. 2012 Aug [cited 2018 Nov 20];18(8).

28. Gama E, Madan J, Langley I, Girma M, Evans D, Rosen S, et al. Economic evaluation of a shortened standardised treatment regimen of antituberculosis drugs for patients with multidrug-resistant tuberculosis (STREAM): study protocol. BMJ Open. 2016;6(10):e014386.

29. Bloemberg GV, Keller PM, Stucki D, Trauner A, Borrell S, Latshang T, et al. Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. N Engl J Med. 2015 Nov 12;373(20):1986–8.

30. Hoffmann H, Kohl TA, Hofmann-Thiel S, Merker M, Beckert P, Jaton K, et al. Delamanid and Bedaquiline Resistance in *Mycobacterium tuberculosis* Ancestral Beijing Genotype Causing Extensively Drug-Resistant Tuberculosis in a Tibetan Refugee. Am J Respir Crit Care Med. 2016 Feb;193(3):337–40.

31. Dara M, de Colombani P, Petrova-Benedict R, Centis R, Zellweger J-P, Sandgren A, et al. Minimum package for cross-border TB control and care in the WHO European region: a Wolfheze consensus statement. Eur Respir J. 2012 Nov;40(5):1081–90.

Annex 1

Zimbabwe National TB Drug Resistance Survey Report, 2016

September 2017

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List of Abbreviations

AIDS	Acquired immune deficiency syndrome
BRTI	Biomedical Research and Training Institute
CDC	U.S. Centers for Disease Control and Prevention
CI	Confidence interval
CPC	Cetyl-pyridinium chloride
D.O.T.	Directly observed treatment
DOTS	WHO-recommended TB control strategy trademark formerly known as Directly observed therapy, short-course
DRS	Drug resistance survey
DSM	Direct smear microscopy
E	Ethambutol
EPTB	Extra-pulmonary TB
EQA	External quality assurance
FDCs	Fixed-dose combinations
GF	Global Fund for the Fight against AIDS, Tuberculosis and Malaria
H	Isoniazid
HBC	High burden country
HIV	Human-immunodeficiency virus
KNCV	KNCV Tuberculosis Foundation
M&E	Monitoring and evaluation
MDR-TB	Multi-drug resistant tuberculosis
MOHCC	Ministry of Health and Child Care
MTB	*Mycobacterium tuberculosis*
NMRL	National Microbiology Reference Laboratory
NTBRL	National TB Reference Laboratory
NTP	National TB Programme
PEDCO	Provincial Epidemiology and Disease Control Officer
PIN	Patient Identification Number
PITC	Provider Initiated Testing and Counseling

PMDs	Provincial Medical Directors
PMDT	Programmatic management of drug resistant tuberculosis
PTB	Pulmonary TB
R	Rifampicin
RHC	Rural health center
SNRL	Supra-national Reference Laboratory of Antwerp, Belgium
TB	Tuberculosis
WHO	World Health Organization
ZIMSTAT	Zimbabwe National Statistics Agency
XDRTB	Extensively drug resistant tuberculosis
Z	Pyrazinamide
ZN	Ziehl-Neelsen

FOREWARD

Zimbabwe has made significant strides over the years in the fight against Tuberculosis (TB). This has been against a backdrop of a generalized HIV epidemic, the most important risk factor for TB in our setting. The response to the HIV has been equally ambitious and aggressive with universal access to antiretroviral therapy within reach in the not distant future.

The gains in the national response to TB continue to be undermined by the increasing burden of Drug Resistant-TB (DR-TB). The last TB Drug Resistance Survey (TB-DRS) was done more than two decades ago and much has changed in the epidemiological landscape to justify this current TB-DRS. The findings of this important undertaking comes on the heels of a recent review of the national TB strategy and will refine target setting within the context of the post-2015 end TB agenda.

The country continues to benefit immensely both financially and technically from development and implementing partners. On behalf of the Ministry of Health and Child Care (MoHCC), I would like to sincerely express our gratitude and appeal for the continued support, and more partners to join us in this fight.

Every stakeholder is implored to reflect on the content of this report, particularly the implications thereof, as we mobilize the much needed resources to consolidate past gains and accelerate our current efforts in this fight.

Brigadier General (Dr) G. Gwinji
Permanent Secretary
Ministry of Health and Child Care

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The piecing together of this report, would have been an up-hill task had it not been for the concerted efforts from various players under the coordination of the AIDS and TB Unit of the MoHCC. Equally it would have been very difficult for the MoHCC to pull this through without the financial and technical support from Challenge TB, a welcome USAID funding mechanism towards the national response to the dual TB-HIV epidemic.

While it may not possible to individually recognize everyone who was involved in this undertaking; special acknowledgements go to all health facilities that participated in this survey, and most importantly, the patients who volunteered their time to participate as consenting respondents during the year-long exercise. Also special technical expert consulted during the survey and putting together this report. Last but not least is the core writing team that synthesized inputs from key stakeholder engagements and refined the final report. Special mention goes to the World Health Organization (WHO), the International Union against Tuberculosis and Lung Disease (The Union), the College of Health Sciences of the University of Zimbabwe for leading the writing process through Dr Joconiah Chirenda and the Zimbabwe Statistical Office (ZIMSTATS) for providing the much needed technical support throughout this undertaking.

Dr. Gibson Mhlanga
Principal Director Preventive Services
Ministry of Health and Child Care

EXECUTIVE SUMMARY

Zimbabwe successfully conducted the 2nd national anti-Tuberculosis Drug Resistance Survey (TB-DRS) in 2015-16. The main objective of this survey was to determine the prevalence of rifampicin resistant and Multi-drug resistant tuberculosis (RR/MDR-TB) among pulmonary smear positive TB patients. Probability proportional to size (PPS) cluster sampling was used to sample 80 of the 146 diagnostic sites that were functional in 2012 and an additional 20, sampled through simple random sampling from 56 sites that became functional by 2014 from which participants were drawn. All sampled sites were public health facilities from all eight rural provinces and two metropolitan cities in the country.

The prevalence of MDR-TB was 1.8% among new cases. This estimate has remained unchanged compared to the last survey conduct two decades ago when the point estimate was 1.9%. The prevalence among retreatment cases declined by close to 4% point, from 8.3% in 1994-5 to 4.6% in this survey. On the contrary, the rate of any rifampicin resistance among new cases doubled from 1.9% in the previous survey to 4.2% in this survey. Of note, no rifampicin mono-resistance was found in the previous survey compared to this survey where half of the reported RR-TB cases was due to rifampicin mono-resistance only. Notably, the country has just begun implementing a policy on use of Xpert MTB/RIF as initial test for all presumptive TB clients, a step in the right direction to ensure early diagnosis and prompt treatment initiation to curb primary transmission.

Being less than 15 years of age; being HIV positive among all cases and history of travel outside Zimbabwe for a period more than a month among new cases were risk factors for RR-TB. Attaining at least secondary education was protective among all cases while participants with history of previous TB treatment were three and half times more likely to have RR-TB.

The algorithm used in the survey where only rifampicin resistant samples by Xpert MTB/RIF proceeded to culture and DST due to resource constraints limited this survey to measure the prevalence of isoniazid mono-resistance, reported as 1.6%

in the previous survey. This is important, considering the on-going national scale up of Isoniazid Preventive Therapy (IPT) for people living with HIV.

The observed risk for RR-TB among children less than 15 years is against the backdrop of under diagnosis of childhood TB in programmatic settings and demands further interrogation, as the sampled participants under represented the much younger age-groups, who present with paucibacillary TB, likely to have been excluded in the survey.

The TB-HIV co-infection rate among the participants in this survey was 55% compared to 65% in the previous survey. This might reflect the observed decline in HIV incidence in the general population over the years.

The prevalence of 2nd line drug resistance among MDR-TB cases was evaluated in this survey with four out of 25 (16%) having additional flouroquinolone resistance and of these, only one (4%) with extensively drug-resistant TB (XDR-TB). Notably, the country only recently begun testing all RR-TB patients with Line Probe Assay (LPA) to rule out second-line resistance in preparation for introduction of shorter MDR-TB treatment regimen and regimens containing new drugs.

Findings from this survey are important and will inform future planning to improve the quality of care for patients diagnosed with DR-TB in Zimbabwe. The following recommendations are proposed for consideration by the National TB Control Programme (NTP): The need to;

- Strengthen implementation of current diagnostic algorithm on universal Drug Susceptibility Testing (DST) for all Xpert confirmed MTB to determine the prevalence of isoniazid mono-resistance, which could not be ascertained in this survey.

- Strengthen laboratory capacity to perform universal DST on all confirmed MTB

- Adopt the use of mobile technology used during the DRS to strengthen dissemination of laboratory results and improve patient management

- Commission future studies on childhood MDR-TB and risk of MDR-TB among migrant populations
- Motivation of health care workers in future DRS to optimize smooth implementation of survey activities.

INTRODUCTION

Global Tuberculosis Epidemiology

According to World Health Organization (WHO) in 2015, there were an estimated 10.4 million new (incident) TB cases worldwide. People living with HIV accounted for 1.2 million (11%) of all new TB cases. There were an estimated 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among people living with HIV. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of deaths worldwide.[1]

Multidrug-resistant TB (MDR-TB) is defined by resistance to both rifampicin and isoniazid, the two core drugs used in the treatment of TB. In 2015, there were an estimated 480 000 new cases of MDR-TB and an additional 100 000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment.[2]

Extensively drug-resistant TB (XDR-TB) is MDR-TB that has developed further resistance to both key groups of second-line therapy, including the fluoroquinolones (e.g. moxifloxacin) and injectable agents (e.g. kanamycin), resulting in fewer therapeutic options, and an increased probability of a fatal outcome. An estimated 9.7% of people with MDR-TB have XDR-TB and XDR-TB has been reported in 105 countries by 2015.[2]

Regional context of the Tuberculosis Epidemic

In 2015, 1.3 million TB cases were notified out of an estimated 2.7 million cases in the WHO African Region. The proportion of TB cases living with HIV was highest in the Region (31%), and exceeded 50% in parts of southern Africa.[1] Member states of the Southern African Development Community (SADC) have continued to bear the brunt of the TB epidemic, mainly on account of the disproportionate burden of HIV. Five countries in the region are among the top 14 high TB burden countries for TB, TB-HIV and MDR-TB, namely Angola, the Democratic Republic of the Congo (DRC), Mozambique, South Africa and Zimbabwe.[1] A notable milestone in the region was the signing by Heads of States of the “SADC Declaration of TB in the Mining sector”, an endorsement of a shared

[1] Global TB Report 2016, WHO
[2] Definitions and reporting framework for tuberculosis - 2013 revision (updated December 2014), WHO

regional commitment to curb the spread of TB among mine workers. The rates of TB in the mines within the region are disproportionately higher than in the general population, with rates in some member states as high as four times the national rates.[3]

The WHO African region confirmed 26 929 MDR/RR-TB cases out of an estimated 42 000 cases in 2015, and an additional 1 100 XDR-TB cases. In the last few years, all member states of the SADC, except Seychelles have reported a case of MDR-TB. Notably, South Africa confirmed as high as 19 613 MDR/RR-TB and 1 024 XDR-TB cases in 2015 (>90% of confirmed cases in the WHO African region), a concern, given its proximity to Zimbabwe and the associated cross border migration dynamics.[1] At least 23 countries in Africa and Asia had introduced shorter regimens for treatment of MDR-TB or RR-TB (17 of which are from the region) by 2015.[1]

Zimbabwean situation of the Tuberculosis Epidemic

Burden of disease: Zimbabwe is among the 30 high burdened countries for TB, TB/HIV and MDR/RR-TB. In the year 2014, Zimbabwe successfully conducted the first National TB Prevalence Survey. The results of which noted a prevalence for all forms of TB of 292 per 100,000 population.[4] The 50 year time series show an initial stable TB case notification of 4000 cases per year (about 100 per 100,000 population), for more than two decades. In the early 90s however, there was a very steep surge, fuelled by the HIV epidemic. Subsequently, there has been a sustained decline in tandem with a progressive increase in the coverage of anti-retroviral treatment (Figure 1).[5]

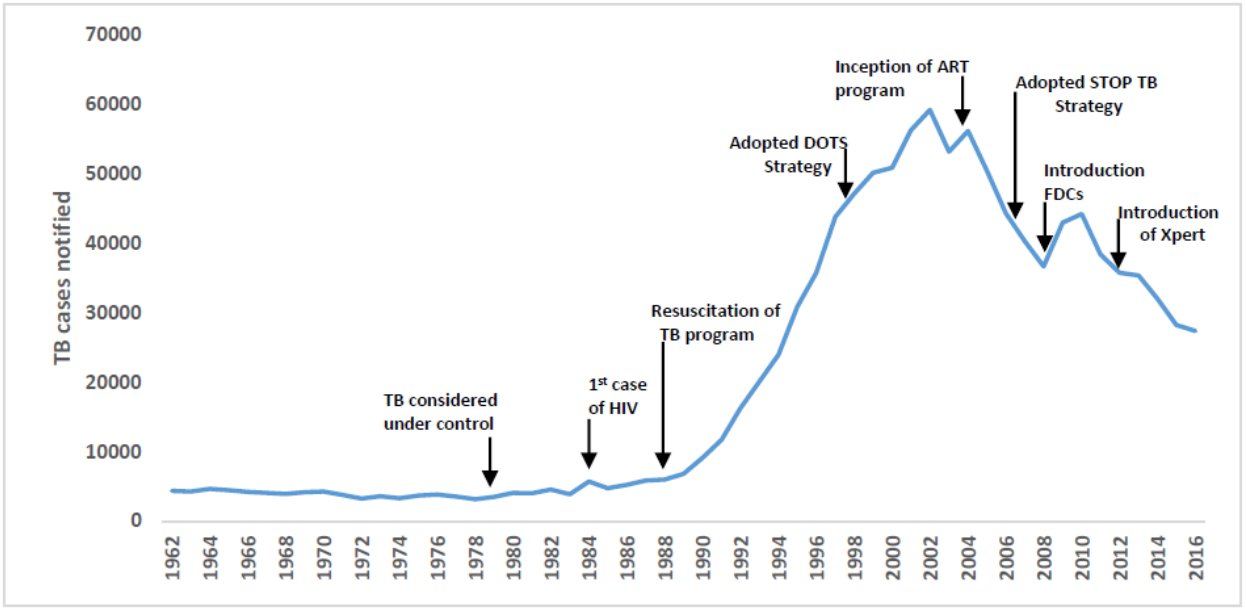
TB-HIV context: Like many such countries in Southern Africa, TB in Zimbabwe has been fueled by HIV with an estimated prevalence of 14.6% among adults aged 15-64 years in 2015.[6] More than two-thirds (72% in 2015) notified TB patients were co-infected with HIV. The rate is much high among notified DR-TB clients (85% for the period 2011-2016 for 4 province from which reported data could be verified). Mortality due to TB alone has shown a slight decline from the rate of 18 per 100,000 population in 2000 to 11 per 100,000

[3] SADC Declaration of TB in the mining sector, 2012
[4] The Zimbabwe National Population Based TB Prevalence Survey, 2014
[5] Epidemiological and impact assessment report, Zimbabwe 2013
[6] Zimbabwe Population Based Impact Assessment (ZIMPHIA) 2015-2016

population in 2015. However, HIV associated TB mortality rates have significantly declined from a peak of 158 per 100,000 population in 2006 to 40 per 100,000 population.[1]

National TB response: Most importantly, the observed decline in TB case notifications over the years can be attributed to an unflinching government commitment to fight the TB epidemic, as demonstrated by the adoption of the Directly Observed Treatment Short Course Strategy (DOTS) in the late 90s the global Stop TB Strategy in 2008. In addition, government embraced a policy of provision of free TB treatment and has deliberately decentralized TB services to the most peripheral public health entity within the health care delivery system. Over the years, there has been a steady expansion in the number of diagnostic centers to more than 220 sites across the country, including two TB Reference laboratories as at December 2016.[7]

Figure 7: Case notification trends (all forms of TB) 1963-2016



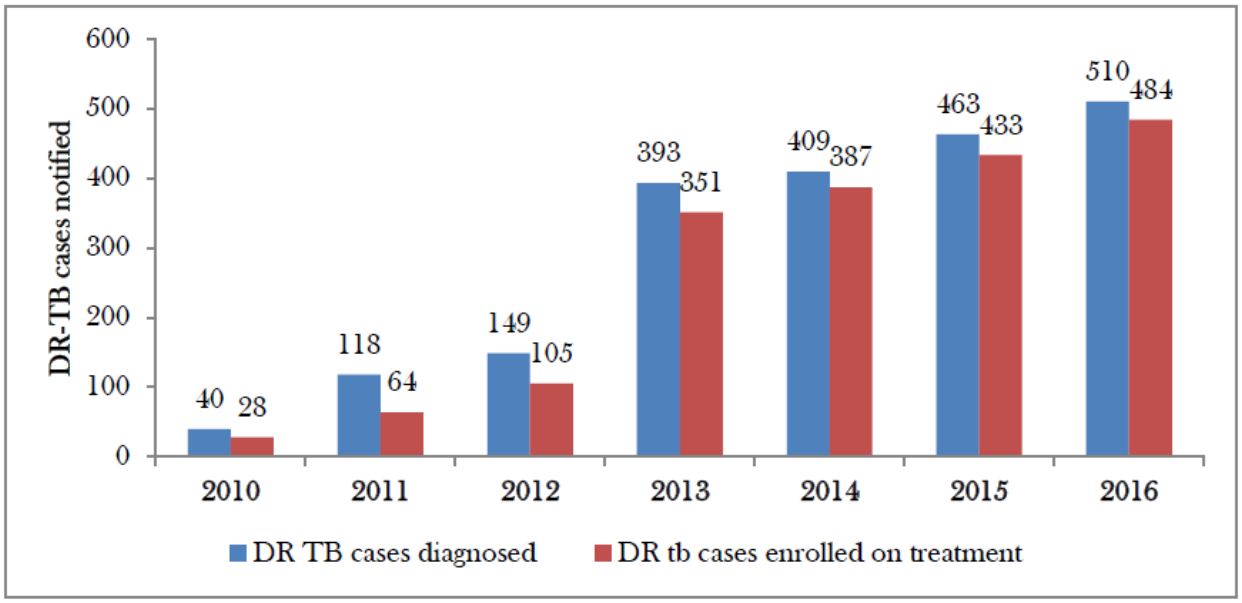
Drug Resistant TB in Zimbabwe

The country embraced Programmatic Management of Drug Resistant TB (PMDT) in 2010 that has seen a progressive improvement in the capacity to detect and treat Drug Resistant TB (DR-TB) and decentralization of PMDT to district level with scale up of more

[7] National Tuberculosis Program –Strategic Plan (2017-2020)

rapid molecular Xpert MTB/Rif technology.[7] With roll out of Xpert MTB/Rif technology, since 2012 (121 machines across all districts as at December 2016), case notification of DR-TB patients has continued to increase annually (Figure 2). Training of Health Care Workers (HCWs) on both PMDT and clinical management of DR-TB has ensured better coordination and decentralization of treatment initiation to district level.[6]

Figure 8: DR-TB cases notified and initiated on treatment in Zimbabwe (2010-2016)



The country has two TB Reference laboratories, the National TB Reference Laboratory (NTBRL) that services the Southern region and the National Microbiology Reference Laboratory (NMRL) servicing the Northern region. Routinely, resistant profiles are ascertained by phenotypic Drug Susceptibility Testing (DST) for all presumptive DR-TB

clients and diagnostic Rifampicin resistant samples by X-pert. Between 2009 and 2016, a total 993 diagnostic samples had successful growth on culture and underwent DST. Among these, 77% were either MDR-TB or Rifampicin mono-resistant. The proportion on INH mono-resistance was 8%.

Table 1: Routine data on resistance profile for DST results, from the two TB Reference laboratories, NTBRL & NMRL (2009-2016)

Resistance profile	*2009	2010	2011	2012	2013	2014	2015	2016	Total
Rif mono Resistance	1	7	24	30	29	26	36	38	191
Isoniazid mono resistance	3	3	7	35	6	9	9	12	84
MDR	6	23	132	162	90	104	55	70	659
Poly resistance	1	0	12	29	13	14	1	10	79
Other mono resistance	1	1	-	16	5	7	-		30
Total DSTs	**12**	**34**	**175**	**272**	**143**	**160**	**118**	**130**	**1,043**

*Results only from one laboratory, NTBRL

1.2 Rationale for the current Drug Resistance Survey (DRS)

The last TB DRS was conducted in 1994 and noted a prevalence of MDR-TB among new cases of 1.9% and 8.3% among retreatment cases. [8, 9] Much has changed since the last survey with scale up of diagnostics and decentralization of both PMDT and clinical management of DR-TB. Routine laboratory surveillance data that could potentially be used as a proxy to track trends in resistance is incomplete, and does not disaggregate by treatment history. The need to assess the current status of drug-resistant TB in Zimbabwe was thus seen as an important priority to better inform resource mobilization and more targeted programming. Additionally, there was a need to determine not only the MDR-TB prevalence, but also that of XDR-TB, an important emerging public health threat in this part of the world, likely to undermine past gains if left unchecked.

[8] Global TB Report 2015, WHO
[9] Anti-Tuberculosis drug resistance in the World, WHO/IUATLD

2.0 SURVEY OBJECTIVES

2.1 General Objective

- To determine the national prevalence of TB drug resistance among pulmonary smear positive TB patients in Zimbabwe.

2.2 Specific Objectives

- To determine the prevalence of Rifampicin resistance among new and previously-treated pulmonary sputum smear-positive TB patients.
- To determine the prevalence of resistance to the other 1st line anti-TB drugs (isoniazid, ethambutol and streptomycin) among new sputum smear positive and previously treated sputum smear positive TB patients with Rifampicin resistance.
- To determine the proportions and pattern of drug resistance to fluoroquinolones and second line injectables among strains with confirmed resistance to Rifampicin and with confirmed, MDR-TB
- To measure the associations between RR/MDR-TB and selected demographic characteristics, including HIV status, sex, age, and other risk factors.
- To evaluate the feasibility of utilizing Xpert MTB/RIF technology (e.g., genotypic vs. phenotypic DST) within the context of a periodic nationally representative drug resistance survey in Zimbabwe.

3. METHODOLOGY

3.1 Study design

- The study was a cross-sectional survey of patients with pulmonary, sputum smear-positive TB (new and previously-treated patients).

3.2 Study setting

- The study was conducted in selected TB diagnostic sites in the public health sector.

3.3 Study population

All sputum positive TB patients who were diagnosed at the selected TB diagnostic sites in Zimbabwe between August 2015 and September 2016.

3.4 Study inclusion criteria

All new and retreatment cases from selected TB diagnostic sites

i) with sputum smear-positive TB

ii) who were not on anti-TB therapy at the time of enrollment.

iii) and consented to participate in the survey

3.5 Sample Size calculation and sampling criteria

New patients: Based on 2012 reported data of 12,405 new sputum smear-positive patients and assuming an MDR-TB prevalence of 1.9% among new cases (based on 1994 Zimbabwe TB-DRS estimates), and a precision of 1% at 95% confidence interval (CI), a sample size of 677 patients was required for a standard non-cluster sample. Applying a design effect adjustment of two for a cluster design and accounting for possible losses of up to 20%, a minimum sample size of 1,625 new smear-positive patients was required.

Retreatment patients: Based on 2012 data of 1,745 sputum smear-positive retreatment patients, assuming an MDR-TB prevalence of 8.3% (based on 1994 Zimbabwe TB-DRS estimates), and a precision of 3% at 95% CI, a sample size of 274 patients was required for a standard non-cluster sample. Applying a design effect of two due to the cluster design and accounting for possible losses of up to 20%, a minimum sample size of 658 retreatment smear-positive patients was required.

The sampling criteria involved 2 stages: selection of TB diagnosing centres in the first stage and then patient recruitment at the second stage. In the first stage, site selection was conducted based on two methods. First, probability proportional size (PPS) sampling was used to select 63 TB diagnostic sites, out of 146 sites that were functional in 2012 and second, an additional 20 TB diagnostic sites were selected from the 56 sites that were previously non-functional in 2012 but later became functional in 2014.

In the second stage, for each selected diagnostic site, consecutive eligible patients were included in the survey until the required number of new cases for that site was reached or up to the end of the survey period. Due to the anticipated slow recruitment of the targeted number of previously treated cases, once the targeted number of new cases per site was achieved, recruitment of patients was stopped.

3.6 Survey management, processes and procedures

To ensure smooth implementation and provisions of continuous technical support, multi-sectorial survey management and steering committees were established. The NTP provided leadership and co-ordination functions. Pre-survey visits were conducted to assess the capacity and readiness of the sites to start recruitment of participants. Survey sites were regularly supervised to support survey implementation including replenishment of stocks.

Three months prior to commencement of the survey, pilot testing was conducted on 10% of the sites which participated in the survey, for up to 8 weeks. This was done in order to evaluate the processes involved in the survey and helped in the identification and resolution of potential bottlenecks. Findings of the pilot informed key changes to the methodology namely; centralization of Xpert diagnosis to the NTBRL, use of Cetylpyridinium chloride (CPC) to preserve samples in transit and modifications to bar codes used in the survey.

Three teams from the national office were identified and trained as trainers. The trainers then trained participating site teams in different provinces on-site. The facility based onsite training was over two days upon which sites started participant enrolment. The facility teams that were trained were health workers responsible for the TB clinics and

laboratory staff. Training started with low volume sites in rural provinces and ended with the two largest urban cities of Bulawayo and Harare and these two had high volume sites. During enrolment, a clinical questionnaire was administered to all consenting participants, followed by collection of two sputum samples. All the samples including the clinical questionnaire were sent through a courier service to the NTBRL.

3.7 Laboratory procedures

To ensure quality sputum collection, two spot sputum samples were collected from participants in separate 50 ml falcon tubes containing 5ml of CPC by a trained health worker**.** These samples once collected were then stored at room temperature before dispatch to the National TB Reference Laboratory (NTBRL). Prior to transportation to the NTBRL, samples were triple packaged to minimize spillage and contamination. To minimize transcription errors a barcode system was used to identify each pair of samples and forms from the diagnosing centers.

For all patients, the two samples were pooled, then vortexed before testing for *mycobacterium tuberculosis* and rifampicin resistance using Xpert MTB/RIF (Cepheid, Sunnyvale CA). In the case of an error in the first Xpert MTB/RIF test result, the remaining sample was centrifuged and then a 2nd Xpert test was done from the deposit. For Xpert MTB+/RIF resistant sample, culture was conducted using Lowenstein Jensen solid media (LJ) while an aliquot was sent to a Supranational TB Reference Laboratory (SRL) in Antwerp for external quality control (EQA).

For all MTB positive isolates identified, both First-line (streptomycin, isoniazid, Rifampicin and ethambutol) and second line (Kanamycin, Amikacin, Ofloxacin, Moxifloxacin, Capraeomycin) phenotypic drug sensitivity testing (DST) were performed on LJ using the proportion method. After the DST, isolates were stored in 10% glycerol at – 20 C for re-culture purposes by the NTBRL and EQA at SRL.

For samples whose cultures failed to grow, Line Probe Assays (LPAs) were conducted at NTBRL to assess for isoniazid resistance and resistance patterns to 2nd line TB medicines. If there was discordance between the Xpert MTB/RIF rifampicin resistant

results and 1st line phenotypic DST, DNA sequencing was conducted at the SRL as a tie breaker.

3.8 Data management and analysis

Data management: A survey register with demographic, clinical and laboratory variables was maintained by each recruiting site. Each recruited patient was assigned a unique Personal Identification Number (PIN) which was linked to the survey register and all data collection tools. Results from the NTBRL for Xpert MTB/RIF, culture and DST were reported back to their respective sites for clinical management of patients and to the NTP for survey data capture.

Data quality was ensured through training of survey teams and scheduled support and supervisory visits. During these visits, the original forms were cross-checked and corrections made. Facility data were checked before transmission to the next level, and double entered into a Census and Surveys Processing System (CSPro) database. A standardized computerized algorithm identified missing or invalid data. Security of data was ensured through storage on a single password protected computer as well as backed up Compact Disks stored in a locked file cabinet. Source documents were also secured in locked file cabinets.

Data analysis: Data were compiled, cleaned/validated, and analyzed using SPSS® software, version 20 (Chicago, Illinois). Adjustment for sampling error due to combining two sample methods and capping of intake at 12 months was made possible by weighted analysis using exact sampling probabilities that were obtained from the numbers of patients registered in the participating clusters only. Prevalence and 95% confidence intervals were calculated for drug resistance among new and retreatment groups.

Analysis of drug resistance patterns, risk factors and demographic characteristics were stratified by new and previously treated patients. Univariate odds ratios and 95% CI were calculated to determine factors associated with Rifampicin resistance and MDR patterns. Stepwise logistic regression was used to assess potential risk factors for multi-drug resistance.

3.9 Ethical considerations

The study protocol, was approved by the Medical Research council of Zimbabwe. All eligible participants in the study provided a written informed consent/assent prior to enrollment and collection of samples.

4.0 RESULTS

During the survey period, a total of 5,279 sputum smear positive patients were notified from the respective clusters. A total 1,301 new and retreatment cases were recruited into the survey. Of these 1,114 (65.5%) out of a target of 1,700 new patients were recruited (Table 1). From the 100 sampled clusters, 86 managed to recruit at least a single patient. A total of 53 clusters managed to recruit at least 14 (80%) of the targeted new patients. A total of 29 participants were excluded from the analysis due to missing survey forms and/or incorrect barcoding. Participants from urban clusters made up to 60.1% (n=765) of surveyed participants. There were variations in enrollment by province ranging from 46.5% (n=166) in Harare to 95.3% in Bulawayo.

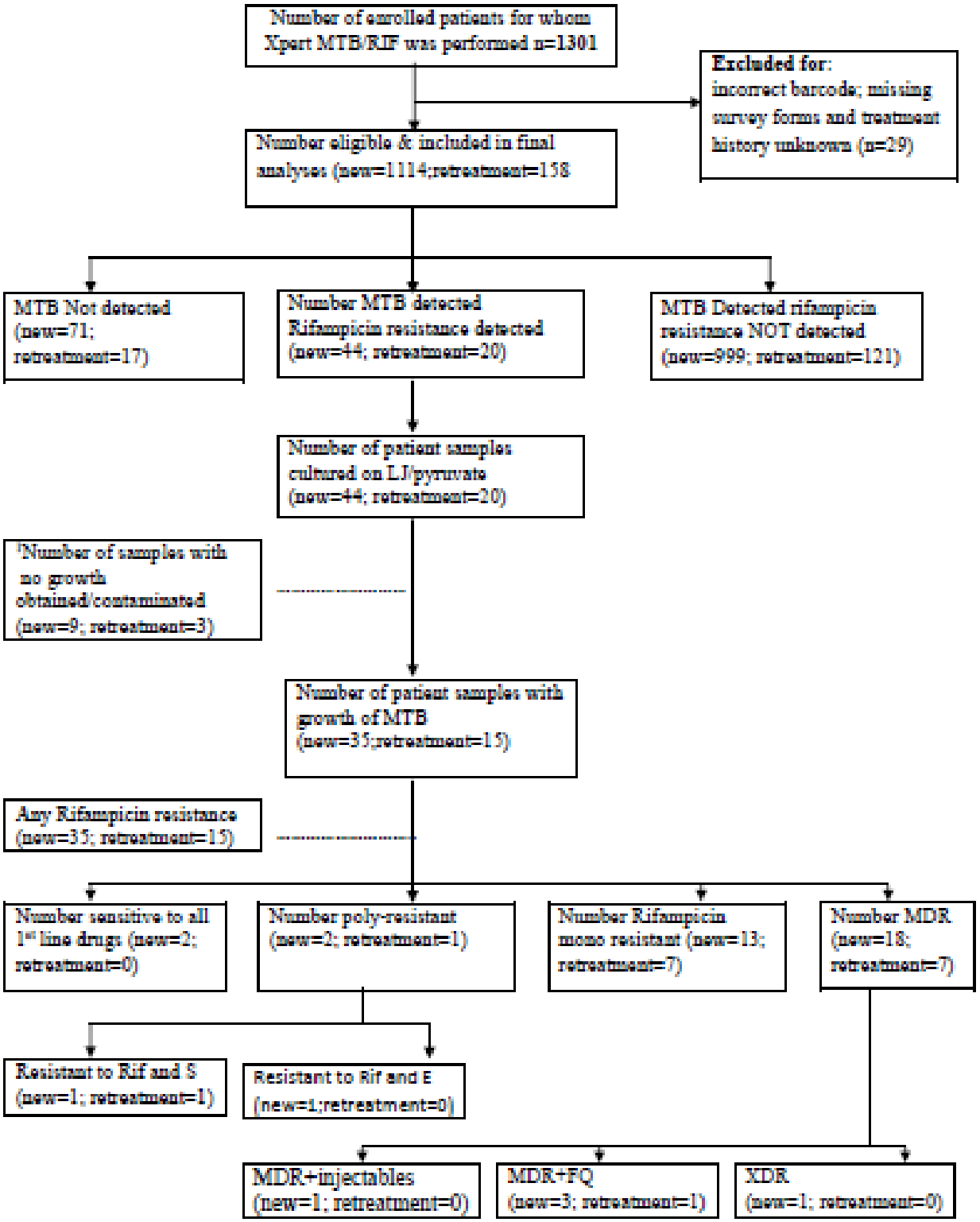
Table 2: Enrollment and participation rate by province, TB-DRS 2015/16

Province	Total SS+ notified during the survey period in the cluster sites	Number (%) of new eligible SS+ patients enrolled in the DR survey	Number of new patients expected
Manicaland	298	135 (79.4)	170
Mashonaland Central	250	138 (73.8)	187
Mashonaland East	304	78 (65.5)	119
Mashonaland West	282	133 (65.2)	204
Matabeleland North	152	71 (54.9)	153
Matabeleland South	328	83 (46.4)	119
Midlands	300	136 (88.9)	153
Masvingo	258	93 (60.8)	153
Harare	2,879	166 (46.5)	357
Bulawayo	228	81(95.3)	85
Total	**5,279**	**1,114 (65.5)**	**1,700**

Among the 1,272 enrolled patients, 1,114 (87.6%) were new while 158 (12.4%) were retreatment cases. All the enrolled patients had an Xpert MTB/RIF assay done with 1,184 (93.1%) confirmed to have mycobacterium tuberculosis (MTB). Xpert MTB/RIF positivity was 93.6% among the new sputum smear positive participants compared to 89.3% in the retreatment group. A total of 64 (5.4%) had rifampicin resistant strains, among whom 44

(68.8%) were new and 20 retreatment cases. Out of the 64 with rifampicin resistant TB strains, 50 (78.1%) successfully grew MTB on LJ. Twelve samples did not grow and two were contaminated. First and second-line phenotypic DST confirmed rifampicin resistance in 48 (96%), with 25 (52.1%) having both rifampicin and isoniazid resistance (MDR) and one with additional resistance to both flouroquinolones and aminoglycosides (XDR) (Figure 3)

Figure 9: Flowchart of all patients enrolled in the Zimbabwe TB-DRS survey (2015-16)



The proportion of patients with rifampicin resistant strains was 4.2% and 14.2% amongst new and retreatment patients respectively (Table 3).

Table 3: Proportion of Xpert MTB+ among smear positive patients by treatment history, Zimbabwe TB-DRS 2015/6

Xpert MTB/RIF result	New n =1,043 (%)	Retreatment n = 141 (%)
MTB+/RIF-	999 (95.8)	121 (85.8)
MTB+/RIF+	44 (4.2)	20 (14.2)

First and second-line phenotypic DST confirmed any rifampicin resistance (RR-TB) in 48 (96%) out of the 50 that grew MTB. The remaining 2 cultures were susceptible to all first line drugs. Among confirmed RR-TB, 20 (41.7%) were rifampicin mono-resistance. A total 25 (52.1%) had both rifampicin and isoniazid resistance (MDR-TB), of which 18 (72%) and 7 (28%) were new and retreatment patients respectively. Three (6.3%) had poly-resistance which all included rifampicin. Among the MDR-TB, one (4%) had additional resistance to both flouroquinolones and aminoglycosides (XDR) (Table 4).

Table 4: Phenotypic resistance patterns by treatment history, Zimbabwe TB-DRS 2015/6

Resistance pattern	New	Retreatment	Total
First-line			
Any Rifampicin resistance	33 (68.8)	15 (31.2)	48
Mono-Isoniazid resistance	0	0	-
Mono-rifampicin resistance	13 (65)	7 (35)	20
*Any poly-resistance**	2 (66.7)	1 (33.3)	3
MDR-TB	18 (72)	7 (28)	25
Second-line			
MDR-TB with DST results for any fluoroquinolone (FQ) & 2nd line injectable (2LI)	18 (72.0)	7 (28.0)	25
MDR-TB susceptible to both FQ & 2LI	13 (68.4)	6 (31.6)	19
MDR-TB with any resistance to FQ	4 (80.0)	1 (20.0)	5
MDR-TB with any resistance to 2LI	2 (100.0)	0 (0.0)	2
MDR-TB patients with any resistance to both FQ & 2LI (XDR-TB)	1 (100.0)	0 (0.0)	1

4.1 Demographic characteristics of enrolled participants

There were 766 (60.2%) male patients who participated in the survey. About 65% of the participants were aged between 25 and 44 years and 19 (1.5 %) were children under the age of 15 years. The median age of the participants was 34 years (IQR 27-42 years). A total of 675 (54%) participants were married and 862 (67.7 %) had completed at least secondary education. Of all survey participants, 765 (60.1%) were from urban clusters (Table 5).

A total of 66 (5.2%) enrolled patients were working in the mining and/or quarry industry, 271 (21.3%) were in the agricultural sector and 10 (0.8%) were health care workers. Among all the enrolled patients tested for HIV, 699 (55%) were positive and 47 (73.3%) among those with rifampicin resistance. The proportion of diabetes among the patients was 1.3%. History of smoking, TB contact in the previous two years and being a miner or former miner were reported by 353 (27.7%), 319 (25.1%) and 200 (15.7%) respectively. A total of 293 (23%) participants had history of any travel of at least a month duration outside the country. Patients reporting travel to South Africa were more than those to any other country (Table 6).

Table 5: Demographic characteristics of eligible new and retreatment sputum, smear positive cases enrolled, Zimbabwe TB-DRS 2015/16.

Demographic characteristics	Total number of smear positive patients enrolled (n = 1,272) Number (%)	New sputum smear positive enrolled (n =1,114) Number (%)	Retreatment sputum smear positive enrolled (n = 158) Number (%)
Sex			
Male	766 (60.2)	668 (60.0)	98 (62.0)
Female	506 (39.8)	446 (40.0)	60 (38.0)
Age group			
<15	19 (1.5)	18 (1.6)	1 (0.6)
15-24	184 (14.5)	171 (15.4)	13 (8.2)
25–34	458 (36.0)	415 (37.3)	43 (27.2)
35-44	365 (28.7)	315 (28.3)	50 (31.6)
45-54	149 (11.7)	116 (10.4)	33 (20.9)
55-64	53 (4.2)	46 (4.1)	7 (4.4)
≥65	42 (3.3)	31 (2.8)	11 (7.0)
Unknown	2 (0.2)	2 (0.2)	0 (0)
Marital status			
Never married	251 (19.7)	229 (20.6)	22 (13.9)
Married/in union	675 (53.1)	600 (53.9)	75 (47.5)
Divorced/separated	210 (16.5)	177 (15.9)	33 (20.9)
Widowed	111 (8.7)	89 (8.0)	22 (13.9)
Unknown	25 (2.0)	19 (1.7)	6 (3.8)
Level of education			
None	43 (3.4)	39 (3.5)	4 (2.5)
Primary	358 (28.1)	312 (28.0)	46 (29.1)
Secondary	794 (62.4)	700 (62.8)	94 (59.5)
Tertiary	68 (5.3)	55 (4.9)	13 (8.2)
Missing	9 (0.7)	8 (0.7)	1 (0.6)
Cluster location			
Urban	765 (60.1)	671 (60.2)	94 (59.5)
Rural	507 (39.9)	443 (39.8)	64 (40.5)

Table 6: Characteristics of enrolled participants by treatment history, Zimbabwe TB-DRS 2015/6

Characteristics	Total number of smear positive patients enrolled (n = 1,272) Number (%)	New sputum smear positive enrolled (n =1,114) Number (%)	Retreatment sputum smear positive enrolled (n = 158) Number (%)
Employment industrial/sector			
Social services	96 (7.5)	81 (7.3)	15 (9.5)
Mining and quarrying	66 (5.2)	60 (5.4)	6 (3.8)
Transportation and couriers	64 (5.0)	59 (5.3)	5 (3.2)
Agriculture, forestry and fishing	271 (21.3)	245 (22.0)	26 (16.5)
Manufacturing and construction	88 (6.9)	75 (6.7)	13 (8.2)
Wholesale and retail trade	241 (18.9)	206 (18.5)	35 (20.3)
Other service activities	102 (8.0)	88 (8.0)	14 (8.9)
Not in employment/Inactive	229 (18.0)	202 (18.1)	27 (17.1)
Not Stated/Unknown	115 (9.0)	98 (8.8)	17 (10.8)
HIV status			
Positive	699 (55.0)	580 (52.1)	119 (75.3)
Negative	526 (41.4)	492 (44.2)	34 (21.5)
Unknown	47 (3.7)	42 (3.8)	5 (3.2)
Risk factors			
Diabetes mellitus	17 (1.3)	15 (1.)	2 (1.3)
Immuno-suppressive therapy	37 (2.9)	30 (2.7)	7 (4.4)
Smoker	353 (27.8)	318 (28.5)	35 (22.2)
Health care worker	32 (2.5)	28 (2.5)	4 (2.5)
Miner or former miner	200 (15.7)	182 (16.3)	18 (11.4)
Homeless	24 (1.9)	21 (1.9)	3 (1.9)
Prisoner in the last 5 years	67 (5.3)	61 (5.5)	6 (3.8)
Contact with TB patient in last 2 yrs	319 (25.1)	288 (25.9)	31 (19.6)
History of any travel			
Outside Zimbabwe for ≥1 month	293 (23.0)	243 (21.8)	50 (31.6)
South Africa	198 (15.6)	166 (14.9)	32 (20.3)
Other SADC countries	79(6.2)	62(5.6)	17(10.8)
Other countries outside SADC	9 (0.7)	8 (0.7)	1 (0.6)
Unknown	7 (0.6)	7 (0.6)	0 (0.0)

4.2 Factors associated with rifampicin resistance

Among all respondents, age less than 15 years, (OR: 6.9, 95% CI: 1.80, 26.45), being HIV positive, (OR: 2.64, 95% CI: 1.46, 4.78), history of traveling outside Zimbabwe, (OR: 1.74, 95% CI: 1.02, 2.97) and history of previous TB treatment (OR: 3.75, 95% CI: 2.14, 6.58) were associated with rifampicin resistant TB infection. Having attained at least secondary education, (OR: 0.58, 95% CI: 0.34, 0.97) had a protective effect against rifampicin resistant (Table 7).

Table 7: Univariate analysis of risk factors for Rifampicin resistance among ALL enrolled patients, Zimbabwe TB-DRS, 2015/6

Risk Factors	MTB+/ Rif+ N=64	MTB+/Rif – N=1120	OR (95% CI)
Sex			
Female	22 (4.7)	444 (95.3)	Reference
Male	42 (5.8)	676 (94.2)	1.25 (0.74, 2.13)
Age group			
<15	4 (22.2)	14 (77.8)	6.90 (1.80, 26.45)*
15-24	7 (4.0)	169 (96.0)	Reference
25–34	19 (4.4)	412 (95.6)	1.11 (0.46, 2.70)
35-44	24 (7.1)	313 (92.9)	1.85 (0.78, 4.39)
45-54	5 (3.6)	123 (96.4)	0.91 (0.28, 2.92)
55-64	3 (6.2)	45 (93.8)	1.61 (0.40, 6.47)
≥65	2 (5.9)	32 (94.1)	0.51 (0.30, 7.60)
Level of education			
Primary and less	27 (7.4)	336 (92.6)	Reference
Secondary and above	36 (4.4)	777 (95.6)	0.58 (0.34, 0.97)
Unknown	1 (12.5)	7 (87.5)	1.78 (0.21, 14.99)
Cluster location			
Urban	38 (5.3)	676 (94.7)	Reference
Rural	26 (5.5)	444 (94.5)	1.04 (0.62, 1.74)
HIV status			
Negative	15 (3.0)	493 (97.0)	Reference
Positive	47 (7.4)	585 (92.6)	2.64 (1.46, 4.78)*
Unknown	2 (4.5)	42 (95.5)	1.60 (0.35, 7.25)
Risk factors			
Diabetes mellitus	0 (0.0)	16 (100)	N/A
Immuno-suppressive therapy	3 (8.8)	31 (91.2)	1.73 (0.51, 5.81)
Smoker	13 (3.9)	323 (96.1)	0.63 (0.34, 1.17)
Health care worker	1 (3.7)	26 (96.3)	0.67 (0.09, 5.00)
Miner or former miner	9 (4.7)	184 (95.3)	0.83 (0.40, 1.71)
Prisoner in the last 5 years	3 (4.7)	61 (95.3)	0.85 (0.26, 2.80)
Contact with TB patient in last 2 yrs	15 (4.9)	289 (95.1)	0.88 (0.49, 1.59)
History of any travel			
Outside Zimbabwe for ≥1 month	22 (7.8)	259 (92.2)	1.74 (1.02, 2.97)*
South Africa	17 (8.9)	173 (91.1)	1.55 (0.55, 4.36)
Other SADC countries	21 (7.8)	249 (92.2)	0.84 (0.10, 6.91)
Treatment history			
New	44 (4.2)	999 (95.8)	Reference
Retreatment	20 (14.2)	121 (85.8)	3.75 (2.14, 6.58)*

Among the new cases, the risk of having rifampicin resistant strains was sustained in age groups less than 15 and 35-44 (OR 11.43 95% CI 2.11, 62.00 and 3.47 95% CI: 1.01, 11.95 respectively). In addition, being HIV positive had about two and half times risk of having rifampicin resistant strains (OR 2.40 95% CI 1.23, 4.73). Having attained at least secondary education remained protective as was for all respondents (OR 0.50 95% CI 0.27, 0.93). There were no differences across gender, being a miner and history of any travel outside Zimbabwe with being rifampicin resistance among both treatment groups (Annex 1 and 2).

After multivariate analysis, age group less than 15 years remained a risk factor (OR 8.59 95% CI 1.47, 50.04) among all cases, while being HIV positive among all and new cases remained significant (OR 2.13 95% CI 1.13, 4.00 and 2.19 95% 1.07, 4.46) respectively. Any travel outside Zimbabwe among new cases was associated with rifampicin resistance (OR 2.05 95% CI 1.07, 4.46). Patients with history of previous TB treatment were three and half times more likely to have rifampicin resistance (OR 3.44 95% CI 1.89, 6.26). Attaining at least secondary education also remained protective among all cases (OR 0.56 95% CI 0.31, 0.99) (Table 8)

Table 8: Multivariate comparison between TB cases with Xpert rifampicin-resistant results compared to TB cases with Xpert rifampicin-susceptibility results by treatment history

Risk factors	Total (n=1184)		New TB cases (*n =1043*)		Retreatment TB cases (*n = 141*)	
	ORadj	95% CI	ORadj	95% CI	ORadj	95% CI
Sex						
Female	1.37	0.78, 2.41	1.08	0.55, 2.12	2.16	0.68, 6.82
Male		Reference		Reference		Reference
Age group						
<15	6.37	1.51, 26.87*	8.59	1.47, 50.04	N/A	
15-24		Reference		Reference		Reference
25–34	0.96	0.38, 2.42	1.62	0.45, 5.84	0.28	0.05, 1.48
35-44	1.29	0.51, 3.23	2.44	0.68, 8.77	0.29	0.06, 1.40
45-54	0.52	0.16, 1.75	0.64	0.10, 4.06	0.19	0.03, 1.11
55-64	1.04	0.24, 4.42	2.43	0.45, 13.27	N/A	
≥65	0.90	0.16, 4.98	1.91	0.19, 19.80	0.20	0.02, 2.56
Level of education						
Primary and less		Reference		Reference		Reference
Secondary and above	0.56	0.31, 0.99*	0.52	0.27, 1.02	0.75	0.21, 2.67
Unknown	2.83	0.30, 27.08	5.11	0.51, 51.25	N/A	
HIV status						
Negative		Reference		Reference		Reference
Positive	2.13	1.13, 4.00*	2.19	1.07, 4.46*	1.76	0.44, 7.09
Unknown	1.34	0.29, 6.24	0.89	0.11, 7.25	2.72	0.21, 34.71
History of any travel						
Outside Zimbabwe for ≥ 1 month	1.69	0.95, 2.99	2.05	1.05, 4.03*	1.09	0.37, 3.16
Treatment history						
New		Reference				
Re-treatment	3.44	1.89, 6.26*				

4.3 Prevalence of RR/MDR-TB

The crude prevalence of RR-TB was 4.2% (95% CI: 3.1 - 5.6) and 14.2% (8.9 - 21.1) among new and retreatment cases respectively, while the crude prevalence for MDR-TB was 2.0% (95% CI: 1.25, 3.06) and 6.4% (95% CI: 2.4-10.3) among new and retreatment cases respectively. The weighted RR prevalence was 4.6% (95% CI: 3.0, 6.2) among

new cases, while that of MDR-TB among new cases was 1.8% (95 % CI: 1.0-2.5). (Table 9)

Table 9: Prevalence of RR-TB by treatment history, all 87 clusters

Statistic	New			Retreatment		
	Number of cases	RR-TB %	95% CI	Number of cases	RR-TB %	95% CI
Individual sampling un-weighted	1,043	4.2	3.1 - 5.6	141	14.2	8.9 - 21.1
Individual sampling weighted for new cases only	1,043	4.6	3.2 - 6.5	141	14.2	8.9 - 21.1
Standard logistic regression - weighted for new cases only	1,043	4.6	3.0 - 6.2	141	14.2	8.5 - 19.9
Standard logistic regression - weighted for new cases only and robust SE	1,043	4.6	3.0 - 6.2	141	14.2	8.9 - 19.5

Table 10: Prevalence of RR-TB by treatment history, with clusters reaching at least 45% of the target cluster enrolment (n=71)

Statistic	New			Retreatment		
	Number of cases	RR-TB %	95% CI	Number of cases	RR-TB %	95% CI
Individual sampling un-weighted	994	4.1	3.0 - 5.6	132	14.4	8.9 - 21.6
Individual sampling weighted for new cases only	994	4.1	3.1 - 5.5	132	14.4	8.9 - 21.6
Standard logistic regression - weighted for new cases only	994	4.1	3.0 - 5.3	132	14.4	8.4 - 20.4
Standard logistic regression - weighted for new cases only and robust SE	994	4.1	3.0 - 5.3	132	14.4	8.9 - 19.9

Table 11: FINAL MODEL: All clusters-this model (standard logistic regression SE) below is the final selected because it is the model which fits the data and allows maximum use of data

Statistic	New		Retreatment	
	No. of cases	RR-TB % 95% CI	No. of cases	RR-TB % 95% CI
Standard logistic regression - weighted for new cases only and robust SE	1043	4.6 (3.0 - 6.2)	141	14.2 (8.9 - 19.5)

5.0 DISCUSSION

Participant enrollment in this survey was comparable to a similar survey done in Lesotho[10] (67%) but lower than those done in Botswana[11], Vietinam[12] and South Korea[13]. Human resource challenges associated with such large scale surveys as reported in similar surveys were also experienced in some sites.[2]

More males participated in the survey, in line with routine data and the Zimbabwe population based TB prevalence survey, where more males were recruited[14]. This is also consistent with surveys done in countries in the region like Botswana (52%) [3], Lesotho (57.5%) [2] and Namibia[15] (55%). Majority of the participants were in the 25 to 44 years age group which is consistent with routine TB programme notification data.[16] The high adult literacy level in the country of 96.7% may explain the observed higher proportion among the participants having completed at least secondary school education. The PPS sampling strategy used, selected more urban clusters than rural, likely because they report more TB cases.[17]

The prevalence of MDR-TB found in Zimbabwe was lower than reported in other SADC countries such as Lesotho (3.1% in new and 12.8% in retreatment cases) [2], Namibia (3.8% in new and 16.5% in retreatment cases) and comparable to South Africa (2.1% in new and 4.6% in retreatment cases)[18]. The RR prevalence observed was almost double that of MDR-TB, consistent with the findings from Lesotho (6.2% in new and 19.5% in retreatment cases) and South Africa (3.4% in new and 7.1% in retreatment cases). The RR-TB prevalence from the survey mirrored the WHO estimates for Zimbabwe (3.2 in new and 14.0 in retreatment cases).[1] A few XDR-TB cases have been reported in Zimbabwe in the last three years which is consistent with only one case found during this survey.

[10] Maama-Maime et al. Anti-Tuberculosis resistance survey in Lesotho, 2008/9: Lessons learned
[11] Menzies et al. Increase in anti-tuberculosis drug resistance in Botswana: results from the fourth National Drug Resistance Survey
[12] Nhung et al. The Fourth National Anti-Tuberculosis Drug Resistance Survey in Viet Nam
[13] Ho Young Lee et al. Drug-resistance pattern of Mycobacterium tuberculosis strains from patients with pulmonary and extrapulmonary tuberculosis during 2006 to 2013 in a Korean tertiary medical center
[14] Zimbabwe population based TB prevalence survey 2014/5
[15] The report of 2008/2009 National Tuberculosis drug resistance survey, Namibia
[16] Zimbabwe NTP Annual Report, 2016
[17] 2014 Zimbabwe Labour Force Survey
[18] South African Tuberculosis Drug Resistance Survey 2012–14

The overall HIV positivity among the survey participants was lower than reported routinely (69%).[11] This is likely a result of excluding smear negative and extra pulmonary TB patients more likely to be HIV positive. The reported positivity however is higher than reported in a similar survey in Namibia (46.3%) and lower than in South Africa (63.2%). However, the positivity among those with rifampicin resistant strains was much higher, consistent with the routine data.

Among new cases, the significant risk factors for being rifampicin resistant TB were age and being HIV positive. The observed increased risk in the age group below 15 years could point to ongoing community transmission. These findings are consistent with what has been reported in South Africa and Namibia.[15, 18] After controlling for HIV infection, the risk of rifampicin resistance in those less than 15 years remained significant. Further studies with larger samples are needed to confirm these findings. The observed increased risk in the 35-44 years age group could have been confounded by HIV status, as this was not sustained in the multivariate analysis. Risk of rifampicin resistance among new patients was high in those that are HIV positive. This remained significant after controlling for potential confounders. This observation is expected considering the high TB-HIV co-infection rate in Zimbabwe. This findings however are contrary to a systematic review of drug resistant TB prevalence and associated risk factors in Sub Saharan Africa (SSA), which showed no significant association between MDR-TB and HIV infection[19]. Countries from the Southern African region that have done drug resistant TB prevalence surveys did not report the association between HIV infection and prevalence of MDR-TB.[10,11,15,18] History of traveling outside Zimbabwe remained a risk factor for being rifampicin resistant among new patients even after controlling for potential confounders. Migration has been confirmed as a known risk factor for drug resistant TB transmission in high and low-income countries[20, 21]. As expected, patients with a history of retreatment had an increased risk of drug resistant TB. History of previous treatment is a known risk

[19] Lukoye et al. Variation and risk factors of drug resistant tuberculosis in sub-Saharan Africa: a systematic review and meta-analysis. BMC Public Health 2015, 15

[20] Pareek et al. BMC Medicine. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review; (2016) 14:48

[21] Cain et al. PLOS Medicine; The Movement of Multidrug-Resistant Tuberculosis across Borders in East Africa Needs a Regional and Global Solution. DOI:10.1371

factor for DR-TB including MDR TB as reported in previous surveys elsewhere and by WHO.[1, 19]

Attaining at least secondary education was protective against being rifampicin resistant TB among new patients. Drug resistant surveys from the SADC region did not assess the relationship between education and risk of drug rifampicin resistant TB. In a study to assess the effect of poverty on risk of TB, low body mass index, associated with poverty was a significant risk factor to TB infection. Although studies from high income countries failed to show the relationship between education and risk of rifampicin resistant TB there is need for further studies to adequately investigate this relationship.

History of working in health care settings, prison and the mining sector were not associated with risk of rifampicin resistant TB in this survey. In other settings, health care workers, miners and prisoners are known risk groups for MDR-TB.[22 , 23]. The relatively small numbers of these risk groups in this survey may explain the inability to demonstrate expected associations. In addition, different study designs used in similar surveys could also explain the difference.

6.0 SURVEY LIMITATIONS AND LESSONS LEARNED

6.1 Survey limitations

Failure to recruit adequate sample size

Some sampled clusters failed to recruit adequate participants due to a combination of factors. These included inclusion of non-functional microscopy sites, staggered recruitment, where some sites recruited for only three months instead of planned twelve months and industrial action by health care workers in some clusters. Challenges related to provision of laboratory consumables also affected recruitment.

Oversampling of urban clusters

[22] Iacopo Baussano et al. Tuberculosis among Health Care Workers; Emerging Infectious Diseases; Vol. 17, No. 3, March 2011
[23] David Stuckler et al. Mining and risk of TB. American Public Health Association. 2011

The two metropolitan cities of Harare and Bulawayo report more smear positive patients than other rural provinces [24]. The programmatic management of drug resistant TB started in Harare and Bulawayo from 2010 to 2013. In addition, these cities had more diagnostic sites per population than rural provinces. Current evidence from the National TB programme show that the top five high MDR-TB high notifying provinces are mainly rural, namely Matebeleland North, Matebeleland South, Bulawayo, Mashonaland Central and Mashonaland East. The use of probability proportional to size (PPS) sampling, sampled more urban than rural clusters. Relatively over sampling of urban clusters based on historic data for drug susceptibility TB could potentially have under estimated the true prevalence of MDR-TB.

Laboratory Procedures

One of our objectives was to assess the feasibility of using Xpert MTB RIF technology within the context of a nationally representative TB DRS. This objective could not be answered because of the diagnostic algorithm used in this survey. Use of culture considered Gold standard in this survey only prioritized Xpert MTB Rif positive and not all samples done Xpert. We also could not estimate the prevalence of INH mono-resistance as not all samples were cultured.

Out of the 64 sputum samples sent for quality control using DNA sequencing, nine did not yield valid results due to either no DNA material or scanty DNA. Preliminary analysis of stored samples on LPA on previously Xpert MTB RIF positive samples have failed to detect MTB. The use of CPC as a preservative may have affected the viability of stored samples.

The ideal procedure for DRS was to transport sputum samples within three days under cold chain to ensure maximum recovery of MTB. In this survey, sputum samples were diluted with 5ml of CPC to 3 ml of sputum. A 2 ml aliquot of study sample at the laboratory may have had a dilution effect on the amount of bacilli in such a way that samples with

[24] Noppert et al. Trends of sputum-smear positive tuberculosis in Zimbabwe: 2008–2011; BMC Res Notes; (2015) 8:575

scanty MTB could have been missed. This observation however calls for further interrogation.

Estimating prevalence of RR/MDR-TB in children under 15 years

Although the inclusion criteria included all age groups, diagnosis of smear positive children under programmatic conditions in Zimbabwe was difficult due to the paucibacillary nature of PTB. As a result, few older children were recruited into the survey. The significant risk of RR/MDR-TB in patients <15 years could not be relied on due to the fewer number of children who were sputum smear positive.

6.2 Lessons Learned

This survey undoubtedly contributed to strengthening of programmatic management of drug resistant tuberculosis (PMDT) through;

Training

- A total of 910 health care workers were trained from 83 sites on research ethics, research methods, laboratory procedures and data management.

Laboratory systems strengthening

- Transportation of samples from peripheral clinics to the national TB reference laboratory (NTRL) used the existing private and public courier system. This system was further strengthened through improved communication between the clinic and the NTRL. Whenever a health facility had samples to send to the NTRL, the facility would call to alert the NTRL. This ensured that all samples were followed up and reached the laboratory.
- A WhatsApp platform was used to disseminate results. This ensured confirmed patients were initiated on the correct treatment regimen timeously. Use of mobile technology could be adopted to disseminate laboratory results, communication of adverse events related to treatment and general coordination of the PMDT.

Patient recruitment

The target of at least 80% sample could not be reached. This was due to resource constraints that could not allow pre-survey assessment of all TB diagnostic sites on capacity of sites to recruit adequate patients for the survey. Mobilization of adequate

resources is essential to ensure successful implementation of such huge national surveys.

Human resources Management

- The survey assumed that selected sites had adequate human resources for survey implementation. At lower level health facilities, existing staff were pulled out of their usual clinical duties during training, disrupting routine facility activities for the two-day training period. Thorough preparation before implementing complicated surveys are required to minimize logistical challenges associated with routine facility activities.
- Motivation of health care workers affected patient recruitment of patients during the period of data collection as most health workers perceived survey activities as an added responsibility.

6.3 CONCLUSIONS

The Zimbabwe TB-DRS represents a reliable point estimate of RR/MDR-TB resistance among new patients. Observed risk factors confirm the contribution of HIV and migration to the MDR-TB epidemic in Zimbabwe. The National TB programme should adopt appropriate policies and programmes to improve MDR-TB prevention and control among high risk groups such as migrants and people living with HIV infection.

RECOMMENDATIONS

Ministry of Health and Child care is encouraged to;

- Develop policies that address the risk of MDR-TB infection among migrant populations like cross border traders and Zimbabweans living in SADC countries as semi-permanent residents
- Leverage current regional initiatives addressing TB and MDR-TB in mining communities and HIV within SADC to minimize duplication of resources for TB control.

National TB Control Programme is implored to;

- Accelerate implementation of the current diagnostic algorithm on universal DST for all Xpert confirmed MTB to determine the prevalence of isoniazid mono-resistance which could not be ascertained in this DRS.
- Strengthen laboratory capacity to perform universal DST on all confirmed MTB
- Adopt the use of mobile technology used during the DRS to strengthen dissemination of laboratory results and improve patient management
- Commission future studies on childhood MDR-TB and risk of MDR-TB in migrants
- Motivate on incentivize health care workers in future TB-DRS to optimize smooth implementation of survey activities

Partners are encouraged to,

- Support resource mobilization for drug resistant TB prevention and control.

References

1. Global TB Report 2016, WHO
2. Definitions and reporting framework for tuberculosis - 2013 revision (updated December 2014), WHO
3. SADC Declaration of TB in the mining sector, 2012
4. The Zimbabwe National Population Based TB Prevalence Survey, 2014
5. Epidemiological and impact assessment report, Zimbabwe 2013
6. Zimbabwe Population Based Impact Assessment (ZIMPHIA) 2015-2016
7. National Tuberculosis Program –Strategic Plan (2017-2020)
8. Global TB Report 2015, WHO
9. Anti-Tuberculosis drug resistance in the World, WHO/IUATLD
10. Maama-Maime et al. Anti-Tuberculosis resistance survey in Lesotho, 2008/9: Lessons learned
11. Menzies et al. Increase in anti-tuberculosis drug resistance in Botswana: results from the fourth National Drug Resistance Survey
12. Nhung et al. The Fourth National Anti-Tuberculosis Drug Resistance Survey in Viet Nam
13. Ho Young Lee et al. Drug-resistance pattern of Mycobacterium tuberculosis strains from patients with pulmonary and extrapulmonary tuberculosis during 2006 to 2013 in a Korean tertiary medical center
14. Zimbabwe population based TB prevalence survey 2014/5
15. The report of 2008/2009 National Tuberculosis drug resistance survey, Namibia
16. Zimbabwe NTP Annual Report, 2016
17. 2014 Zimbabwe Labour Force Survey
18. South African Tuberculosis Drug Resistance Survey 2012–14
19. Lukoye et al. Variation and risk factors of drug resistant tuberculosis in sub-Saharan Africa: a systematic review and meta-analysis. BMC Public Health 2015, 15
20. Pareek et al. BMC Medicine. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review; (2016) 14:48

21. Cain et al. PLOS Medicine; The Movement of Multidrug-Resistant Tuberculosis across Borders in East Africa Needs a Regional and Global Solution. DOI:10.1371
22. Iacopo Baussano et al. Tuberculosis among Health Care Workers; Emerging Infectious Diseases; Vol. 17, No. 3, March 2011
23. David Stuckler et al. Mining and risk of TB. American Public Health Association. 2011
24. Noppert et al. Trends of sputum-smear positive tuberculosis in Zimbabwe: 2008–2011; BMC Res Notes; (2015) 8:575

7.0 ANNEXES

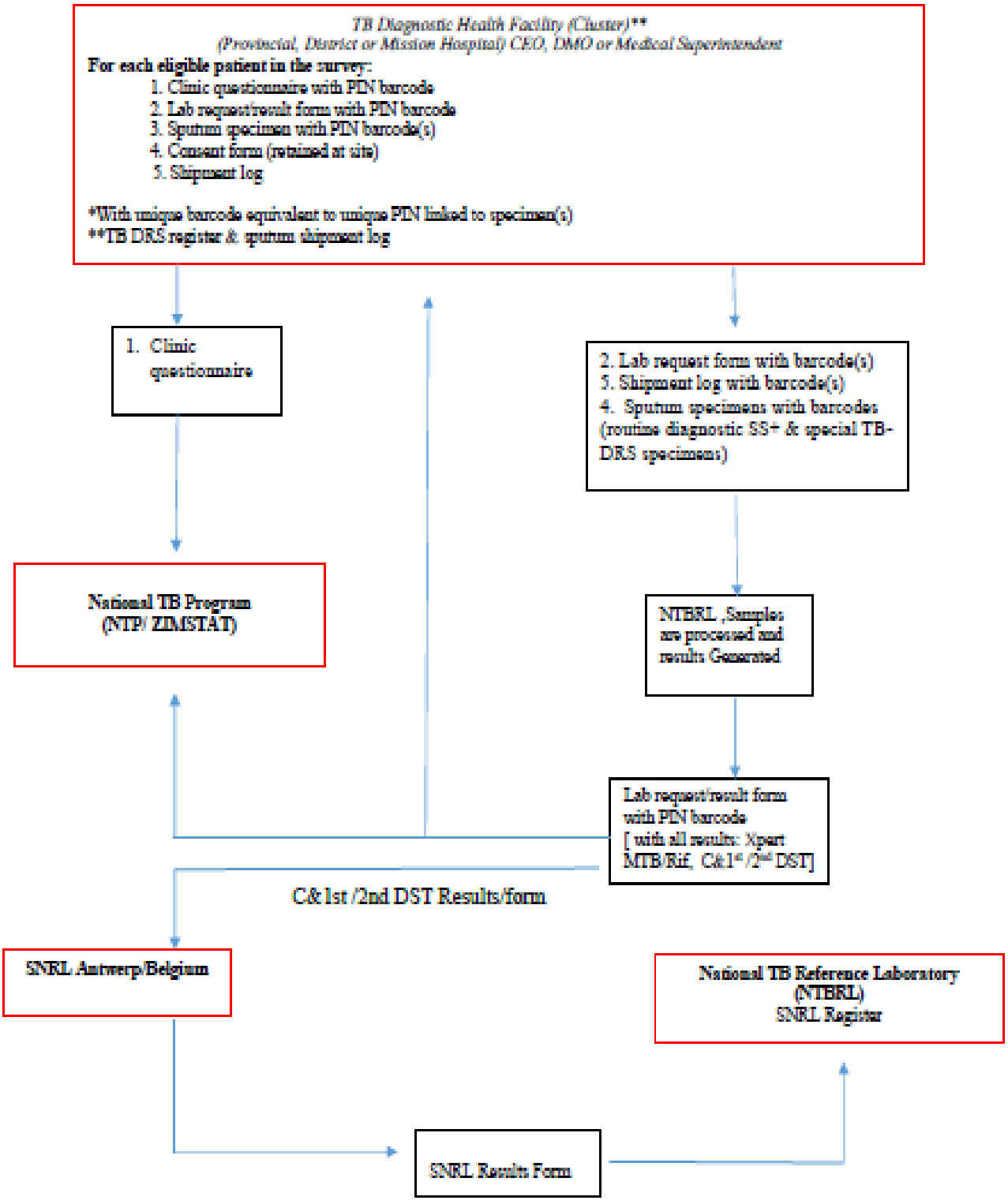
Annex 1: Univariate analysis of risk factors for Rifampicin resistance among enrolled new patients, Zimbabwe TB-DRS, 2015/6

Risk Factors	New patients		OR (95% CI)
	Xpert MTB+/ RIF+ n=20, number (%)	Xpert MTB+/ RIF- n=121, number (%)	
Sex			
Female	17 (4.1)	398 (95.9)	Reference
Male	27 (4.3)	601 (95.7)	1.05 (0.57, 1.96)
Age group			
<15	3 (17.6)	14 (82.4)	11.43 (2.11, 62.00)*
15-24	3 (1.8)	160 (98.2)	Reference
25–34	14 (3.6)	377 (96.4)	1.98 (0.56, 6.99)
35-44	18 (6.1)	277 (93.9)	3.47 (1.01, 11.95)*
45-54	2 (1.9)	106 (98.1)	1.01 (0.17, 6.12)
55-64	3 (7.1)	39 (92.9)	4.10 (0.80, 21.11)
≥65	1 (4.0)	24 (96.0)	2.22 (0.22, 22.24)
Unknown	0 (0.0)	2 (100.00)	N/A
Level of education			
Primary and less	20 (6.2)	302 (93.8)	Reference
Secondary and above	23 (3.2)	691 (96.8)	0.50 (0.27, 0.93)*
Unknown	1 (14.3)	6 (85.7)	2.52 (0.29, 21.93)
Cluster location			
Urban	25 (4.0)	603 (96.0)	Reference
Rural	19 (4.6)	396 (95.4)	1.16 (0.63, 2.13)
HIV status			
Negative	12 (2.5)	463 (97.5)	Reference
Positive	31 (5.9)	498 (94.1)	2.40 (1.23, 4.73)*
Unknown	1 (2.6)	37 (97.4)	1.02 (0.13, 8.02)
Risk factors			
Diabetes mellitus	0 (0.0)	14 (100)	N/A
Immuno-suppressive therapy	1 (3.6)	27 (96.4)	0.84 (0.11, 6.31)
Smoker	10 (3.3)	296 (96.7)	0.70 (0.34, 1.43)
Health care worker	1 (4.2)	23 (95.8)	0.99 (0.13, 7.48)
Miner or former miner	5 (2.8)	172 (97.2)	0.62 (0.24, 1.59)
Prisoner in the last 5 years	2 (3.4)	56 (96.6)	0.80 (0.19, 3.40)
Contact with TB patient in last 2 yrs	12 (4.4)	999 (95.8)	1.05 (0.53, 2.06)
History of any travel			
Outside Zimbabwe for ≥1 month	15 (6.4)	218 (93.6)	1.85 (0.98, 3.52)
South Africa	13 (8.2)	146 (91.8)	3.21 (0.70, 14.59)
Other SADC countries	14 (6.3)	208 (93.7)	0.67 (0.08, 5.64)

Annex 2: Univariate analysis of risk factors for Rifampicin resistance among enrolled retreatment patients, Zimbabwe TB-DRS, 2015/6

Risk Factors	Re-treatment		OR (95% CI)
	Xpert MTB+/ RIF+ n=20, number (%)	Xpert MTB+/ RIF- N=121, number (%)	
Sex			
Female	5 (9.8)	46 (90.2)	Reference
Male	15 (16.7)	75 (83.3)	1.84 (0.63, 5.40)
Age group			
<15	1 (100)	0 (0.0)	N/A
15-24	4 (30.8)	9 (69.2)	Reference
25–34	5 (12.5)	35 (87.5)	0.32 (0.07, 1.45)
35-44	6 (14.3)	36 (85.7)	0.38 (0.09, 1.62)
45-54	3 (10.0)	27 (90.0)	0.25 (0.05, 1.34)
55-64	0 (0.0)	6 (100)	N/A
≥65	1 (11.1)	8 (88.9)	0.28 (0.03, 3.07)
Level of education			
Primary and less	7 (17.1%)	34 (82.9)	Reference
Secondary and above	13 (13.1)	86 (86.9)	0.73 (0.27, 2.00)
Unknown	0	1 (100)	Not applicable
Cluster location			
Urban	13 (15.1)	73 (84.9)	Reference
Rural	7 (12.7)	48 (87.3)	0.82 (0.31, 2.20)
HIV status			
Negative	3 (9.1)	30 (90.9)	Reference
Positive	16 (15.5)	87 (84.5)	1.84 (0.50, 6.76)
Unknown	1 (20.0)	4 (80.0)	2.50 (0.21, 30.22)
Risk factors			
Diabetes mellitus	0 (0.0)	2 (100)	N/A
Immuno-suppressive therapy	2 (33.3)	4 (66.7)	3.25 (0.55, 19.05)
Smoker	3 (10.0)	27 (90.0)	0.61 (0.17, 2.25)
Health care worker	0 (0.0)	3 (100.0)	N/A
Miner or former miner	4 (25.0)	12 (75.0)	2.27 (0.65, 7.91)
Prisoner in the last 5 years	1 (16.7)	5 (83.3)	1.22 (0.14, 11.03)
Contact with TB patient in last 2 yrs	3 (10.3)	26 (89.7)	0.65 (0.18, 2.37)
History of any travel			
Outside Zimbabwe for ≥1 month	7 (14.6)	41 (85.4)	1.05 (0.39, 2.84)
South Africa	4 (12.9)	27 (87.1)	0.69 (0.14, 3.53)
Other SADC countries	7 (14.6)	41 (85.4)	N/A

Annex 5: Flowchart of data collection tools used in the TB DRS



Annex 6: Flowchart of specimen handling at the NTRL